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Electroporation of stomata: Effect on drying kinetics and aroma compounds of Genovese basil leaves

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Abstract

The aroma components of herbs and spices are very sensitive to heat, complicating their drying process. Until now, exporters of dried herbs face the challenge of low total phenolic contents and efforts are constantly being made to improve the drying efficiency of herbs and conserve their aroma. This thesis looks at the influence of extracellular trehalose impregnated by vacuum impregnation (VI) and electroporation achieved with reversible pulsed electric field (PEF) treatment affecting stomata opening on the convective air drying kinetics, rehydration properties, colour changes and aroma preservation of basil leaves. In describing the drying kinetics of treated basil samples, attention is given to the determination of the drying rate and the effective moisture diffusivity.

The results indicate that reversible electroporation of opened stomata resulted in faster drying rate (from 0.025 to 0.00039 min^{-1}) compared to samples with opened stomata not electroporated (from 0.0202 to 0.0021 min^{-1}) throughout the drying period. Vacuum impregnation with trehalose increased the initial drying rate of the control from 0.012 to 0.016 min^{-}but towards the end of drying, the drying rate of the control and vacuum infused control were slightly the same (0.0025 and 0.0024 min^{-1}) respectively. Similar trends were also observed for effective moisture diffusivity where samples with stomatal electroporation resulted in increased effective moisture diffusivity ($1.857*10^{-9}$ m^2 s^{-1}) compared to samples where the opened stomata were not electroporated ($1.309*10^{-9}$ m^2 s^{-1}). As a result of the increased drying efficiency, irreversible electroporation of the leaf tissue resulted in 47% reduction, reversible electroporation of opened stomata resulted in 37% reduction whilst leaves samples that were reversible electroporated but opened stomata was not electroporated resulted in 18% reduction in the drying time. The quality parameters analysed on the dried herb indicate that reversible electroporation of opened stomata resulted in less colour change, produced good rehydration properties and resulted in 1.3 to 5.7 fold increase in the major aroma compounds relative to the untreated dried samples.

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List of Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
D_{eff}	Effective moisture diffusivity
dMR	Change in moisture content
DRC	Dry mass Retention Capacity
dt	Change in time
FC	Fusicoccin
FDA	Flourescein Diacetate
GC-MS	Gas Chromatography-Mass Spectrometry
k	Drying rate (constant)
m/z	Mass/charge
MC	Moisture content (dry basis)
M_e	Equilibrium moisture content
M_o	Initial moisture content
M_R	Moisture ration
M_t	Moisture content at any stage in the drying process
NIST	National Institute of Standards and Technology
PBS	Phosphate buffered saline
PEF	Pulsed Electric fields
PI	Propidium iodide
R^2	Coefficient of determination
RA	Rehydration Ability
RMSE	Root mean square error
SD	Standard deviation
SSE	Sum of square error
t	Drying time
USDA	United States Department of Agriculture
VI	Vacuum impregnation
WAC	Water Absorption Capacity

1. Introduction

Sweet basil (*Ocimum basilicum L.*) is an aromatic herb belonging to the family *Lamiaceae*. The basil plant is cultivated not only for its culinary use which is mostly derived from the leaves but also for its medicinal and antioxidant properties. Fresh basil is used to enhance flavour in many Mediterranean dishes such as tomato products, pizza, salads, soups and marine foods while the dried basil leaves are mostly used in confectionary, bakery and flavoured products. (Simon et al., 1999; Díaz-Maroto, 2004). Fresh basil leaves have high moisture content (92 %) at harvest and cannot be preserved for long at ambient conditions. If marketed in the dried form, the high moisture content of the leaves needs to be lowered to safe level to extend the shelf life of the product. Drying is an old method of food preservation widely used for such purpose. Drying of plant materials induces structural changes which leads to loss of nutritional value, tissue damage and colour change.

Until now, exporters of dried sweet basil leaves face the challenge of low total phenolic contents. This is because the aromatic constituents of herbs and spices are very sensitive to heat, complicating their drying process. Often, the dehydration of herbs and spices is carried out at low temperatures to help preserve most of the volatiles components. A major challenge with the low temperature drying is the slow removal of moisture, leading to long drying times and high energy consumption. The need to reduce the cost of heat treatment coupled with increased consumer demand for processed products that retain most of the characteristics of the original produce has led to the development of various pre-drying techniques aimed at accelerating the rate of moisture removal.

Vacuum infusion (VI) of solutes with known biological membrane preservation properties have been shown to protect cellular tissue during air drying and improve rehydration properties of plant tissue (Ataré et al., 2009; Oliver et al., 2012). Pulsed electric field (PEF) is a non- thermal pre-treatment technique which is receiving considerable attention in drying applications due to its potential to enhance drying efficiency (Ade-Omowaye et al., 2003; Lebovka, 2007). The increased drying efficiency of PEF treated samples is attributed to irreversible plasma membrane disintegration at high field strength as an upstream process to enhance mass transfer (Gachovska et al., 2008; Ade-Omowaye et al., 2003). However, complete cell disruption cannot be used as a pre-treatment to enhance the drying rate of herbs since their aromatic constituents will leak out.

Plants lose water through opened stomata during growth but the stomata gradually closes when a plant is cut. In a recent work (Advance course: Food engineering) on basil leaves, we observed that reversible electroporation of opened stomata at low field strength enhanced the rate of moisture removal during convective air drying of the leaves and the drying time was considerably reduced. The reduction in drying time as a result of opened stomatal electroporation indicates the potential of targeting opened stomata to enhance the rate of moisture removal during dehydration without irreversible damage of cell membranes of the cells in the leaves.

2. Objectives

The aim of the present study is to analyse the effects of stomata opening and closure, reversible PEF treatment affecting stomata opening and extracellular trehalose impregnated by VI on the convective air drying kinetics, rehydration properties and aroma preservation of basil leaves. The specific objectives of the thesis work are:

- Determine the effect of stomatal opening and closure on the convective air drying rate of basil leaves
- Determine the effect of stomatal electroporation on the convective air drying rate of basil leaves
- Determine the effect of VI with trehalose on the convective air drying rate of basil leaves
- Determine the effect of reversible PEF treatment on the rehydration properties of dried basil leaves
- Determine the effect of stomatal electroporation on aroma retention in dried basil leaves
- Determine the effect of reversible PEF treatments on colour change during drying
- Determine the effect of VI with trehalose combined with reversible PEF on the rehydration properties of dried basil leaves

3. Background

3.1. Basil plant

Sweet basil (*Ocimum basilicum L.*) is an aromatic plant belonging to the family *Lamiaceae.* The genus *Ocimum* contains about 50 to 150 species which are either hardy annuals or non-woody perennial herbs and shrubs (Simon et al., 1999). The basil plant can grow up to 20 – 60 cm long depending on the cultivar (Chalchat and Özcan, 2008). Basil is believed to have originated from the tropical and subtropical regions of Asia, Africa and Central and South America (Simon et al., 1999) but is now extensively cultivated in many Mediterranean countries for its culinary use. There are many cultivars of basil with variable leaf shape and size. Different cultivars show variation in morphological features, essential oil production and the composition of the oils (Lachowicz et al., 1997; Miele, 2001). The most commonly cultivated basil cultivars are Genovese, Purple Ruffles, Cinnamon, Globe, Thai, Mammoth and African Blue. Most of these cultivars marketed for their culinary use are cultivars of sweet basil.

3.1.1. Chemical compounds

The presence of essential oils and their composition determines the specific aroma of herbs. The oil yield of herb varies greatly under different environmental conditions. A number of phenyl-propanoids, terpanoids, monoterpenes, sesquiterpene hydrocarbons etc such as 1, 8-cinoele, methy cinnamate, methyl chavical citronellol, linalool, myrcene, ocimene, eugenol and linalool have been noted as main volatiles responsible for the aroma of basil (Simon et al., 1999; Gang et al., 2001; Díaz-Maroto, 2004). As by 1999, approximately 190 chemical compounds had been identified in basil. Cultivars of basil have varying essential oils composition which depends on the growing conditions, method of extraction and analysis (Gil et al., 2002). These chemicals come together in different proportions to produce a distinctive spicy pungent aroma. No particular cultivar contains all of these volatiles in significant amount but there is often one dominant volatile in each cultivar. Sweet basils for example have a strong clove fragrance due to higher proportions of methyeugenol whist the citrus aroma of lemon and lime basils is due to higher proportions of citral (Simon et al., 1999).

3.1.2 Uses

Sweet basil is a popular culinary herb in most countries used extensively as a food ingredient. Fresh basil leaves contain about 92 % water and is a good source of vitamin A, Vitamin C, vitamin K, iron, folate and traces of other vitamins and minerals (Table 1). Dried basil leaves are mostly used as a spice and the basil seed is used in Asian drinks. Genovese basil (a cultivar of sweet basil) is cultivated mainly for its use in the traditional Genovese sauce popularly known as 'pesto'. Extract from basil have been used traditionally to treat headaches, cough, diarrhoea, constipation, warts, worms and kidney malfunction (Simon et al., 1999; Vierira and Simon, 2000). Basil extract contains biologically active constituents that have insecticidal, nematicidal, fungicidal, and antimicrobial and antioxidant properties (Lee et al., 2005; Lee and Scagel, 2009).

Table 1. Nutritional value of fresh basil, per 100g serving, source: USDA, 2014

Proximate	amount	**Minerals**	amount	**Vitamins**	amount
Energy	94 KJ	Calcium	177 mg	Vitamin C	18 mg
Carbohydrate	2,65 g	Iron	3.17 mg	Thiamin	0.034 mg
Water	92.06 g	Magnesium	64 mg	Riboflavin	0.076 mg
Dietary fibre	1.6 g	Phosphorus	56 mg	Niacin	0.902 mg
Fat	0.64 g	Potassium	295 mg	Vitamin B-6	0.155 mg
protein	3.15 g	Sodium	4 mg	Folate	68 µg
		Zinc	0.81 mg	Vitamin A	264 µg
				Vitamin E	0.8 mg
				Vitamin K	414.8 µg

3.2. The physiology for regulating water loss from leaves

3.2.1. Stomatal movement in plants

Stomata are microscopic pores circumscribed by two guard cells which are located in the impermeable cuticle that coats the aerial parts of terrestrial plants. Plants loss about 95 % of the water that is lost during growth through opened stomata. Stomatal opening or closing is controlled by the movement of the surrounding guard cells. The stomata close in response to less efficient carbon fixation and utilization in order to decrease the loss of water through transpiration (Assmann, 1993). The controlled movement of stomatal aperture allows the plant to balance the demand for carbon dioxide and water.

Guard cells movement is also triggered by its turgidity which is dependent on the concentrations of primary osmolytes. It is a well-known fact that increased guard cells osmolarity induces stomatal opening whilst decreased guard cells osmolarity leads to stomatal closure. The increased accumulation of major osmolytes such as K^+, Cl^-, $malate^{2-}$ and sucrose increases the turgidity of guard cells (Talbott and Zeiger, 1998). These accumulations are triggered by light activated plasma membrane H^+-ATPase through different signalling pathways. Talbott and Zeiger (1998) observed a pathway which involves the uptake of K^+ and Cl^- from the apoplast and the synthesis of $malate^{2-}$ from starch-derived carbon skeletons. High K^+ decreases the osmotic potential of guard cells leading to the influx of water into guard cells. This mechanism increases the turgor pressure in guard cells and inflates them into two kidney bean-like shapes thereby opening the stomata in the centre of two guard cells (Johansson et al., 1993; Schroeder et al., 2001; Hlavinka et al., 2013). In a reverse form, stomatal closure is induced by increased efflux of K^+ ions from guard cells, leading to increased membrane depolarization and turgor loss (Schroeder et al., 2001; Negi et al., 2008).

Much is known about the role of hormones on guard cells osmolarity and on stomatal movement. Johansson et al (1993) reported the activation of guard cells plasma membrane H^+-ATPase by the fungal toxin, fusicoccin (FC). Hlavinka et al (2013) reported the opening of stomata treated by FC and/or exposed to white light in their epidermal strip experiment. Irving et al (1992) observed an initial acidification of guard cell cytosol by FC and other hormones prior to stomatal opening and H^+-ATPase activation.

The phytohormone abscisic acid (ABA) is widely known to induce the closure of opened stomata and inhibits the opening of closed stomata through a signalling cascade driven by the efflux of K^+ from guard cells and involving H_2O_2 and Ca^{2+} as intermediary components (Wang et al., 2001). ABA-dependent depolarization of plasma membrane is partly attributed to its ability to inhibit the action of plasma membrane H^+-ATPase (Schroeder et al., 2001). When the roots sense water deficit in the soil, they send a signal to the shoots through increased concentration of ABA in leaf apoplast or guard cells which eventually produces a chemical signal leading to stomata closure (Dodd, 2003).

3.3. Pre-drying treatments

The choice of any pre-drying processing depends on the kind of material to be processed. Many applications such as ultrasound (Carcel et al., 2007), vacuum impregnation and pulse vacuum (Deng and Zhao, 2008; Ataré et al., 200; Oliver et al., 2012), blanching (Akyol et al., 2006) and electric field pulses (Ade-Omowaye et al., 2003) have been used as pre-drying steps to affect tissue integrity and accelerate drying of plant tissues (Gachovska et al., 2008). The effect of VI and PEF treatment on the drying kinetics is reviewed below.

3.3.1. Vacuum impregnation (VI)

VI is reported to improve the quality of dried and frozen products through a cascade of cellular structure modification. VI involves the application of low pressure to provoke the elimination of gas/air in the pores of the product. The eliminated gas is replaced by a surrounding solution with some specific solute components when atmospheric pressure is restored (Fito et al., 2001; Fito and Chiralt, 2003). Mass transfer in the open pores is driven by hydrodynamic mechanism resulting from the applied pressure gradients (Fito and Pastor, 1994). The reduced porosity and incorporated solute might offer some protective action on the cellular structure of the product, thereby changing the mass transfer behaviour.

Disaccharides such as glucose, sucrose and trehalose have been demonstrated to preserve the function of biological membranes in the dry state (Ataré et al., 2009; Oliver et al., 2012) and freeze state (Phoon et al., 2008; Velickova et al., 2013) and have been used for cellular structure modification. Apple samples pre-treated with trehalose were found to shrink less during drying and had better rehydration properties (Ataré et al., 2009; Ataré et al., 2008; Oliver et al., 2012). Ferrando and Spiess (2001) also observed cellular structure protection of trehalose during their work on the osmotic dehydration of onion and strawberry. The extent of vacuum impregnation and tissue deformation are analysed by different methods. However, the most important condition is to ensure maximal mass gain in the infused samples.

3.3.2. Pulsed electric field (PEF)

PEF treatment is a non-thermal food processing technique which involves the application of a high intensity electric field to biological cell membranes. Cell electroporation induces permeabilization on cell membranes, causing the cells to undergo dielectric breakdown and eventual disruption of the cell membrane. Every cell has its threshold electric field strength

beyond which the cell membranes are irreversibly damaged, resulting in cell death. The extent of cell damage is dependent on the relevant electric parameters: field strength, pulse duration, pulse width, number of pulses, treatment time, whether bipolar or monopolar pulses and on product components (Rastogi, 2003; Dymek, 2014a). The degree of tissue damage and cell disintegration is proportional to the field strength, higher electric field leads to greater cell disintegration (Gachovska et al., 2008; Lebovka, 2007).

PEF treatment ensures continuous application with little heating of the medium, the treatment time is short and there is low energy requirement (Barsotti and Cheftel, 1999). The application of PEF to enhance the drying efficiency of various food materials have been reported by many authors. Angersbach and co-workers (1997) reported an increased permeability of potato tissue in response to PEF treatment, resulting in improved mass transfer during fluidized bed drying. Armyanov and co-workers (2001) reported 20-30 % reduction in drying time of PEF treated tobacco leaves. Amami and co-workers (2008) studied the combined effect of PEF and osmotic dehydration pre-treatment on the convective drying of carrot tissue and observed a reduction in the drying time. Gachovska et al (2008) observed an increased drying rate of PEF-treated carrot. Wu and co-workers (2011) studied the influence of PEF pre-treatment on vacuum freeze-drying of apples and reported 22.5 % reduction in the drying time and 27.02 % improvement in the drying rate. Ade-Omowaye and co-workers (2001) reported that high PEF pre-treatment improved the drying rate of paprika.

The net effect of PEF treatment is accelerated moisture removal during further processing leading to reduced processing time and energy cost, and enhanced product quality (Ade-Omowaye et al., 2003; Gachovska et al., 2008). There is an interactive effect of the various PEF parameters on the drying rate and drying time (Gachovska et al., 2008). These benefits of PEF treatment have been achieved through irreversible plasma membrane permeabilization. By strict control of the electroporation parameters, permeabilization may evade affecting the cell viability. The effect of reversible membrane permeabilization on the rate of moisture removal, drying time and rehydration properties has not yet been explored and it is the main aim of this study.

Reversibly electroporation occurs when the applied electric field is below the threshold of the transmembrane potential and pores are created temporarily in the cell membrane. In this case, the pores are able to reseal and the cell membrane completely recover from electroporation thus preserving the viability of the cell membrane (Dymek, 2014a).

Reversibly electroporation is often used in medical applications where substances such as dyes, drugs, proteins and nucleic acids are incorporated into cells (Garcia et al., 2010). A recent application of reversibly electroporation of plant tissue was reported by Phoon et al. (2008). The authors reported improvement of the freezing tolerance of spinach leaves after reversible PEF treatment and vacuum impregnation with trehalose. Dymek and co-workers (2014a) reported that the reversibility of electroporation in rucola leaves was higher for bipolar pulses compared to mono polar pulses, with increased interval between the pulses further enhancing cell viability after electroporation.

3.4. Drying of herbs

Water is an important component of vegetables and the first step in any preservation operation is the removal of moisture (drying). In biological systems such as food, water exists as free or as bound water. Drying of herbs must meet certain requirements (Oztekin and Martinov, 2007):

(1) moisture content that is at equilibrium with storage conditions
(2) Minimum reduction in active volatile components and in colour
(3) Microbial load must be below the prescribed limits
(4) Good rehydration properties

To help achieve these requirements, adequate temperature, air velocity and humidity values coupled with some pre-drying treatments are combined to provide rapid reduction in the moisture content without affecting the colour and composition of the aroma constituents. Many drying techniques have been employed in recent years to help achieve the above requirements. Microwave drying, oven drying, freeze-drying, sunlight drying have specifically been used to dry basil. However, hot air drying is still the most commonly used dehydration method for basil (Chiampo et al., 1997; Di Cesare et al, 2003; Pirbalouti et al., 2013).

3.4.1. Convective air drying of herbs

Air drying of biological tissues results in simultaneously phenomena of mass transfer, structural changes and tissue modification with drying time. The microstructure of plant tissue such as its porosity affects the mass transfer process. At the same time, mass transfer provokes microscopic and macroscopic structural modifications such as destruction of natural

tissue and shrinkage that in turn affect the mass transfer processes (Barat et al., 2001; Fito and Chiralt, 2003; Seguí et al., 2010). The structural modification may lead to shrinkage, texture and colour changes and lose of nutritional value; resulting in low quality dried products.

Two transport processes occur simultaneously during the convective air drying of plant tissues. These processes are heat transfer and moisture movement. Dehydration involves the evaporation of water from a wet surface to a stream of moving air. There is a simultaneous migration of water from the interior of a drying material toward the surface as water is being removed from the surface. The process creates a difference in vapour pressure on the drying surface and the vapour pressure of water in air surrounding the surface (moisture flux) is hence the driving force for moisture removal. The rate of moisture loss is thus proportional to the remained moisture to be lost (Toledo, 1999).

Convective air drying occurs in a number of stages with different drying rates at each of the stages. At the early stages of drying, characterised by a constant drying rate, the transfer of moisture from the centre to the surface is mainly by capillary action. Capillary action is more rapid and the rate of surface evaporation controls the rate of drying. At this stage, the rate of drying is limited by the mode of heat transfer and the rate at which heat is transferred from air to the material (Toledo, 1999; Lewicki, 2006).

In the later stages of drying, characterised by the falling rate period, the diffusion of water from the centre of the solid to the surface controls the rate of drying (Wang et al., 2007). Moisture diffusivity is affected by composition, moisture content and porosity of the drying material (Afzal and Abe, 1998). For high moisture content produce, moisture removal often leads to some physical changes such as shrinkage and cell collapse. These changes lead to decreasing rates of moisture diffusion. Toledo (1999) noted that the rate of moisture diffusion may be constant if cells do not collapse and pack together during drying.

3.4.2. Mathematical modelling of convective air drying

Two main groups of mathematical models are used to describe the thin-layer drying process of agricultural produces. One group of mathematical models correspond to empirical models .Another group of models correspond to diffusion models. These models describe the mass transport process during drying and give mathematical expressions which:

1. Either links the drying rate as a function of time or as a function of moisture content and
2. Relates the total amount of moisture leaving the drying sample to the drying time.

Depending on the type of model, the following parameters: (1) the effects of the thickness of the drying sample, (2) heat and mass transfer, (3) shrinkage variation of the product and (4) the effect of the drying conditions are taken into consideration in the drying simulation.

During drying, the loss in weight of a sample (representing the loss of moisture) is monitored and the drying process is simulated by processing the obtained weight loss data. Curves showing the variation of moisture content (kg water per kg dry weight) with drying times are presented. In drying simulation, the drying curve is normalised by converting the moisture content (kg water per kg dry weight) data to a dimensionless parameter (moisture ratio) and fitted with the drying time. The moisture content, M_t (kg water per kg dry weight) at any time during the drying process is given by an expression of the form:

$$M_t = M_e + (M_o - M_e) f(t, a, b) \qquad \text{Equation [3.1]}$$

An expression for the dimensionless moisture ratio, *MR* at a time,'*t*' during the drying process is obtained from Eqn 3.1 and is given by:

$$MR = \frac{Mt - Me}{Mo - Me} \qquad \text{Equation [3.2]}$$

where M_o is the initial moisture content (kg water per kg dry weight) and M_t is the moisture content at any time in the drying process (kg water per kg dry weight) and M_e is the equilibrium moisture content (kg water per kg dry weight).

For samples with long drying times, the value of equilibrium moisture content is relatively small compared to the initial moisture content, hence equation (3.2) can be simplified to equation (3.3) with negligible error.

$$MR = \frac{M_t}{M_o} \qquad \text{Equation [3.3]}$$

In drying simulation, the *MR* is related to a mathematical function [f (t, a, b)] with 1 or 2 fitting parameters (a and b) as:

$$MR = f(t, a, b) \qquad \text{Equation [3.4]}$$

Drying constant

In the falling rate period, the rate of moisture lost is assumed to be proportional to the moisture remaining to be lost. This assumption is expressed in the form:

$$\frac{dMR}{dt} = -\ k\ (M_o\ -\ M_e) \qquad \text{Equation [3.5]}$$

Integration of this expression yields the Newton or exponential drying model (Eqn 3.6) which is often used in drying simulation to describe the falling rate period (Sarimeseli, 2011).

$$\frac{Mt-Me}{Mo-Me} = exp(-\ k * t) \qquad \text{Equation [3.6]}$$

where *k* is the drying rate (min^{-1}) and *t* is the drying time (min). Fitting the exponential model to the dimensionless moisture ratio vs time curves yields drying rate values at different stages of drying.

Moisture diffusivity

The drying kinetics of biological materials is described using their transport properties (moisture diffusivity, thermal diffusivity, mass transfer coefficients, etc.) and the properties of the drying medium. At the falling rate period, it is assumed that the main mechanism controlling the drying rate is internal moisture diffusivity. The solution for this equation is based on an assumed uniform initial moisture concentration and negligible external gas phase mass transfer resistance. The drying curve at the falling rate period is thus modelled using the diffusion equation (Akpinar, 2005; Mariani et al., 2008). According to the diffusion model, the total amount of moisture leaving an infinite slab with a constant surface flux is related to the drying time (Akpinar, 2006; Sarimeseli, 2011) in the form:

$$MR = \frac{8}{\pi^2}\left[\sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2}\, exp[-\ (2n+1)^2\ D_{eff}\ t(\frac{\pi}{2t})\ 2]\ \right] \qquad \text{Equation [3.10]}$$

For long drying times, only the first term of Eqn 3.10 is relevant and the diffusion model for infinite slabs is modified with little error to:

$$MR = \frac{Mt-Me}{Mo-Me} = \frac{8}{\pi^2} * exp[-\ \frac{\pi^2 * D_{eff}}{4L^2} *\ t] \qquad \text{Equation [3.11]}$$

Where D_{eff} = effective moisture diffusivity ($m^2\ min^{-1}$), L = half thickness of the leaves (m). The effective moisture diffusivity is calculated from the slope derived from the linear regression of the curves obtained by plotting $ln\ [(\frac{\pi^2}{8})\ *\ MR]$ versus time.

3.4.3. Effect of air drying on quality parameters

Texture and rehydration aspects

Structural changes such as cell deformation and shrinkage induced on plant tissues during pre-drying and drying operations may lead to poor rehydration properties. Rehydration is done by immersion in water or other liquids such as oil, fruit juice, and sugar solutions. When a dried product is immersed into a liquid medium, the product imbibes the liquid which leads to swelling of the product and leaching of soluble solids into the liquid (Lewicki, 1998). The rehydration property of dried products that need rehydration prior to consumption is an important quality criterion.

Rehydration properties can be used to quantify the physical and chemical changes suffered by the material during drying (Krokida and Philippopoulus, 2005; Sacilik and Elicin, 2006). If there is no tissue damage, dried products should be able to imbibe and bind the same amount of water evaporated from them, rehydration properties is thus an important quality index. Maldonado et al., (2010) outline the following three methods to characterise rehydration properties of dried products.

1. Water absorption capacity (WAC). This is the ability of the dried matrix to absorb water to replace the water lost during drying
2. Dry mass retention capacity (DRC). This measures the ability of the material to retain soluble solids after rehydration.
3. Rehydration ability (RA). This is a measure of the ability of the product to rehydrate, gives an estimate of the total tissue damage induced by pre-drying and drying operations on the plant tissue.

Flavour and colour aspects

The drying method has significant effects on the quality of dried products. The removal of water during drying reduces the water activity of dehydrated products to levels that prevent microbial growth and reduces the rate of deteriorative chemical and biochemical reactions (Kubra and Rao, 2012). The reduced weight of dehydrated products reduces storage and

distribution costs and extends shelf life (Toledo, 1999). However, exporters of dried herbs and spices still face the challenge of low total phenolic contents. Consumers characterise dried food products based on their colour and texture resemblance to the raw material (Lewicki, 2006). The dehydration process of aromatic plants often leads to some quality deterioration mainly through loss of volatiles and flavours, loss of vitamin C, and colour change (Kostaropoulos & Saravacos, 1995).

The aromatic constituents of herbs and spices are very sensitive to hot air drying conditions. Drying temperature is the single most important parameter which influences the aroma composition and colour change of dried herbs and spices. Higher temperatures may lead to volatilization or degradation of the active ingredients (Venskutonis et al., 1996; Johnson et al., 1998). Changes in the volatile oils composition and the concentration of some specific compounds of herbs during drying have been observed (Dìaz-Maroto et al., 2002). The temperature-sensitivity of the aroma compounds determines the drying temperature.

Often, the dehydration of herbs and spices is carried out at low temperatures to help preserve most of the volatiles components. Chiampo and co-workers (1997) suggested an oven drying temperatures close to 60 ℃ as optimal to conserve most of the volatile compounds in basil. A major challenge with the low temperature drying is the slow removal of moisture, leading to long drying times and higher energy consumption. The choice of the optimal drying temperature is thus a central economic and quality criterion to be considered.

Colour is an important food attribute that influences consumer acceptance. The colour of green leafy vegetables is due to chlorophyllic pigments namely the less stable chlorophyll a and more stable chlorophyll b. chlorophyll a is the major pigment and is blue-green coloured whilst chlorophyll b is yellow-green coloured. As the leaf is heated, chlorophyll is less stable and is easily degraded. Thermal degradation of the chlorophyllic pigments due to the loss of Mg^2 produces a colour change from the bright green colour to the brown colour of pheophytins a and b observed in dried green leafy vegetables (Eskin, 1990; Johnson et al., 1998).

Non-destructive evaluation of colour can be done using the CIELAB colour space (L^*, a^*, b^*). The L^*coordinate is a measure of the colour brightness with a value 0 representing black and 100 representing white. The chromaticity coordinate a^* measures redness when positive and green when negative whilst the chromaticity coordinate b^* measure yellowness when

positive and blueness when negative. Chen (2008) descriptive levels of colour change as determined from colour change (ΔE) values are shown in Table 2.

Table 2: Descriptive levels of colour change, (adapted from Chen (2008)).

Level	**ΔE range**
Trace level difference	0 – 0.5
Slight difference	0.5 – 1.5
Noticeable difference	1.5 – 3.0
Appreciable difference	3.0 – 6.0
Large difference	6.0 – 12.0
Very obvious difference	> 12.0

4. Materials and methods

4.1. Source of basil and sample preparation

Potted Genovese basil (*Ocinum basilicum*) was purchased at a local supermarket in Lund. The potted plants were kept in cooled bags (5 ℃) and transported to the laboratory. To ensure equal period of exposure to light, the plants were stored at 5 °C for 12 h in a dark room before exposing them to white light for 1 h to initially open the stomata. After exposure to light, 2-3 leaves of equal shape were manually detached from each stem and treated as described below.

4.2. Determination of moisture content

Portions of fresh and treated samples were subject to moisture content (wet and dry basis) determination according to the AOAC (1997) official method. Triplicates of 2 g of each sample were dried in a pre-heated oven at 105 °C for 1 h and the loss in weight was expressed as a percentage of the fresh weight.

4.3. Treatments

4.3.1. Influence of stomata on drying times - Chemical incubation with ABA and FC

After the 1 h exposure to light to initially open the stomata, three batches each consisting of 50 leaves (22 - 24 g) were either incubated in solutions of 10 µM ABA (to force stomata closed), 5 µM FC (to force stomata open) for 2 h in the light prior to drying or not incubated. The incubation solutions were enough to completely cover the leaves and the leaves were held in such a way that the cut portion of the leaf petiole was facing downward in the incubation medium. In this way, the incubation solutions were absorbed through the leaf petiole to the leaf veins and other parts of the leaf. The non-incubated batch was stored on moist tissue paper to avoid dehydration of the leaves.

The ABA solution was prepared in PBS buffer, pH 7.4. PBS buffer was prepared by adding 8 g NaCl, 0.2 g KCl, 1.44 g KH_2HPO4 and 0.24 g KH_2PO_4 to 1000 ml deionized water and adjusting the pH with HCl (Dymek et al., 2014b). Ten mM stock solution of FC was prepared by mixing 146.9 µL of 35% ethyl alcohol to 1 mg bottle of FC and mixed until FC was fully dissolved. 50 µL of this stock solution was added to 100 ml deionized water. After incubation, three leaves from each batch were rinsed in deionized water and observed

under a light microscope (Elipse Ti-U, Nikon, Japan). Images were acquired with the help of an equipped camera (Nikon DS- QiMc) equipped with the image analysis software NIS Element BR which allowed for stomatal aperture measurements at 20X magnification. The remaining leaves from each batch were immediately subjected to drying.

4.3.2. Vacuum impregnation

Vacuum impregnation was carried out with aqueous solution of trehalose in a vacuum desiccator at room temperature. Three untreated basil leaves were immersed in 100 ml trehalose solution (4.5 g /100 g) for 30 min. This duration comprised a gradual increase of the vacuum for 10 min, 10 min holding time at 50 mbar followed by a gradual release of the vacuum for 10 min.

4.3.3. PEF treatment

Untreated and vacuum impregnated leaf samples were placed in a rectangular electroporation chamber (0.5 cm gap between electrodes) and PEF treated with either deionized water or propidium iodide (PI) (conductivity 250 µs). The conductivity of treatment solutions was adjusted with NaCl. The treatment solution was enough to completely cover the stainless steel electrodes of the PEF treatment chamber. The electric pulses were delivered by a CEPT pulse generator (Arc Aroma Pure AB, Lund, Sweden) at room temperature and the electric parameters were programmed by a computer software package (ARC CEPT HM13, Lund, Sweden). The PEF treatment system was connected to a digital oscilloscope (Fluke 123, Washington) which allowed for monitoring the pulses delivered to the samples.

Table 3. Combination of pulsed electric parameters

Field strength (KV/cm)	Pulse width (µs)	Pulse space (µs)	Number of pulses
	120	500	65
0.6	150	760	65
1.5	150	760	65

One train of monopolar, rectangular electric pulses with field strengths of 0.6 or 1.5 kV/cm was delivered. After preliminary trials (not shown in this report), the pulse width was set at 120 or 150 µs, and consisted in 65 pulses with 500 or 760 µs resting period between the pulses. The combination of the electric parameters is shown in Table 3.

4.3.4. Drying

Treated samples including the control were dried in a preheated convection oven at 50 °C overnight to establish drying curves. The air velocity in the oven was 2 m/s and the oven drying chamber was equipped with a balance which allowed for continuous weighing of the samples. The loss in weight of the samples (representing moisture loss) was continuously monitored during the drying process. Samples for further analysis were treated and dried to the experimentally determined drying times using the same drying equipment as described above and their moisture contents were determined according to the AOAC (1997) official method. In order to describe the thin layer convective drying kinetics of vacuum infused and PEF-treated basil leave samples, the *MR* obtained from the drying data is related to the Newton and diffusion models and these mathematical expressions were used to evaluate the effect of the treatments on the drying rate and the effective moisture diffusivity respectively.

4.4. Analysis

4.4.1. Mass gain during vacuum impregnation

The mass gain of vacuum infused basil samples was calculated according to the following equation.

$$m_{weight\ gain} = \frac{m - m_o}{m_o} * 100 \qquad \text{Equation [4.1]}$$

where m is the mass of the infused basil leaves and m_o is the initial mass of fresh basil leaves.

4.4.2. Evaluation of PEF treatments

Test of electroporation

PEF-treated samples were observed under the microscope as described above using 250 µM PI as treatment solution. After electroporation, stained sections of the leaf samples were rinsed with deionized water before observing them under epi-fluorescence light conditions. The nuclei of electroporated cells were stained with PI and were easily identified under fluorescence at 10X magnification (Gómez Galindo et al., 2008).

Test of cell viability

PEF-treated samples were placed on a moist tissue paper and stored in the fridge at 4 ℃ for 20 h. The viability of cells 20 h after electroporation was evaluated by comparing them to negative and positive controls. The negative control was prepared by storing fresh leaf samples in the freezer for 10 min. PEF-treated and control samples were incubated in 12 µM Flourescein Diacetate (FDA) solution for 1 h in the dark at room temperature. The FDA solution was prepared by adding 200 µL of 12 mM FDA stock solution in acetone to 200 ml deionized water. After incubation, stained sections of the samples were rinsed with deionized water and the rinsed samples were observed under the microscope as described above. Viable cells were easily identified by a bright fluorescence at 10X and 20X magnifications (Dymek et al, 2014a).

4.4.3. Effect of treatment on colour change during drying

The colour of fresh and dried basil samples was determined by a chroma meter using the hunter colour standards. The chroma meter was calibrated against white light and set to CIE standard. Five leaves from each sample were used for colour measurement and the values reported are means of 10 replicates. The parameters recorded were the L^*, a^*, b^* coordinates of the CIE lab scale. The total colour change (Eqn 4.2), which is a measure of the colour difference between raw (R) and dried (D) basil leaves was calculated to evaluate the effect of the treatments on colour retention (Di Cesare, 2003).

$$\Delta E = [\sqrt{} + (L^*R - L^*D)^2 + (a^*R - a^*D)^2 + (b^*R - b^*D)^2] \quad \text{Equation [4.2]}$$

4.4.4. Rehydration ratio

The rehydration capacity of the samples was determined according to the method described by Gaschovska et al. (2009) with slight modification. Four dehydrated basil leaf samples were weighed and placed in beakers containing 300 ml distilled water at room temperature for seven hours. Samples were taken from the beaker every hour and drained on a mesh for 5 min before weighing. The rehydration capacity was calculated as the ratio of the weight of rehydrated sample during rehydration to the weight of dry sample.

4.4.5. Water activity

The water activity of the dried leaf samples was determined using the Aqualab (Model CX-2, Decagon Devices Inc., Pullman, WA) water activity analyser.

4.4.6. Analysis of volatile compounds

Extraction

Fresh and dried basil leaves were subjected to supercritical fluid extraction. 2 g of fresh basil leaves and equivalent amounts of dried basil samples were calculated to obtain equal amount of dry matter content and loaded into 5 ml stainless steel extraction cell filled with glass beads. The MV-10 ASFE (waters Technologies, Manchester, UK) supercritical fluid extraction system was used for CO_2 expanded ethanol extraction. The system operation and parameters setting were controlled by ChromScope™ Software.

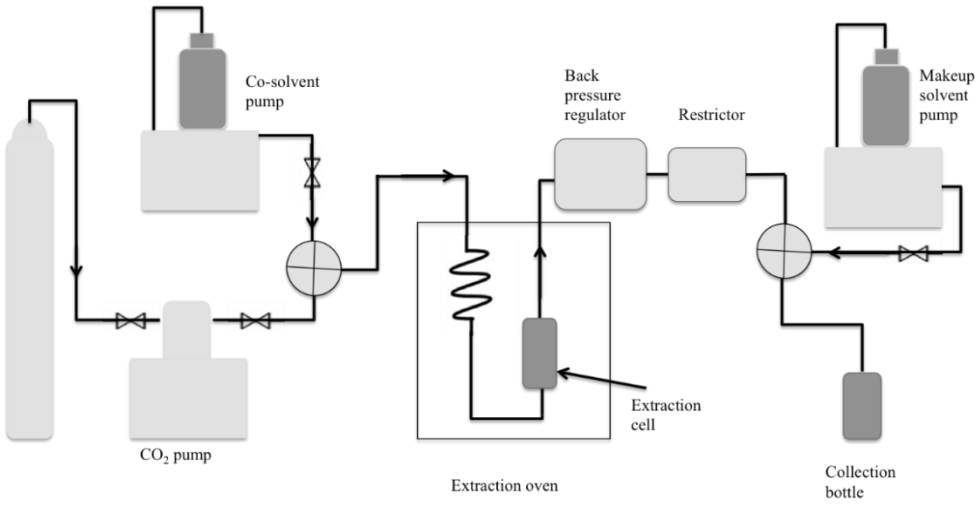

Figure 1. Supercritical fluid CO_2 expanded ethanol extraction system

The system, as illustrated in Figure 1, is equipped with dual piston pumps for CO_2 and co-solvent connected with T joint, extraction oven with 10 extraction cells of 5.0 ml, back pressure regulator (BPR), heated restrictor, make-up solvent pump and 10 bottles collection tray. The extraction was performed at 40 °C, 93 bar and 0.31 molar fraction of CO_2 in ethanol. The flow rate and extraction time were 1.0 ml/min and 60 min respectively. The makeup solvent (ethanol) was kept at 0.3 ml/min. The outlet was bubbled into 5.0 ml of liquid solvent in volumetric flask placed into ice bath. The system was flashed with CO_2/ethanol fluid mixture after each run for cleaning. The extracts were stored at -20 °C until further analysis.

GC-MS Analysis

GC–MS analysis was performed using an 450-GC system (Bruker Corporation, Freemont, CA, USA) equipped with an autosampler and SCION triple quadrupole (TQ) mass spectrometry detector. A Capillary column HP-5 MS fused-silica cap. Column (5% phenyl/95% dimethylpolysiloxane; 30 m x 0.25 mm i.d., film thickness 0.25 µm) was used for analysis. The system was operated at 70 eV ionization energy, 0.5 s/scan and the mass range *m/z* 40 to 350. The oven temperature was held at 60 °C for 3 min then increased to 270 °C at a rate of 3 °C/min, both injector and detector temperatures were set at 250 °C. Helium was used as the carrier gas with a flow rate of 1.0 ml/min. The software adopted to handle mass spectra and chromatograms was SCION SQ Software.

The tentative identification of compounds was done by comparing the obtained mass spectral with those of an internal NIST standard reference mass spectral library and by comparing the results with those reported in literature. The retention of the major aroma compounds in the treated dried samples relative to the untreated dried sample was calculated by dividing the peak area of each compound in the treated samples by their peak areas in the untreated dried sample. Analysis of chemical compounds was repeated three times and mean values$\pm$ standard deviation are reported.

4.5. Statistical analysis

Drying experiments were performed in duplicate and average values were used for curve fitting using Matlab curve fitting toolbox (Matlab R2014b, MathWork, Inc., MA, USA). The goodness of fit of fitted models was evaluated using the coefficient of determination (R^2), the root mean square error (*RMSE*) and the sum of square error (*SSE*). The data were analysed for the degree of variation and significance of difference based on one way analysis of variance (ANOVA) using Microsoft Excel 2013 and Minitab 17. The Turkey's pair-wise comparison test was used to determine the difference ($P < 0.05$) between the treatment means.

5. Results

5.1. Effect of treatments on leaf structure and initial moisture content

Fresh basil leaves have a typical thickness of 0.30 ± 0.02 mm and average weight of 0.41 ± 0.8 g (Table 4). The mass gain in the leaves was 40.46 ± 6.4 %, causing the mean mass of the vacuum impregnated leaf to increase to 0.57 ± 0.1 g. The replacement of the gas in the pores of the leaf structure significantly ($P < 0.05$) decreased the average thickness of basil leaf to 0.25 ± 0.01 mm.

Table 4. Leaf thickness and mass gain after vacuum impregnation with trehalose (n = 20)

Leaf weight (g)			Leaf thickness (mm)	
initial weight	After VI	% mass gain	fresh	After VI
0.41±0.8	0.57±0.1	40.46±6.4	0.30±0.02A	0.25±0.01B

Means ± SD followed by different letters within a row are significantly different (P < 0.05)

The initial moisture content (Table 5), wet basis of fresh basil (control) was 91.20 ± 0.10 %. Both the PEF-treatments and the 2 h incubation of the leaves in the solutions significantly ($P < 0.05$) affected the initial moisture content of the leaves compared to the control. After drying to the predicted drying times, the water activity of all treated dried samples were the same and lower than 0.6 - the limiting value of water activity for the growth of microorganisms (Martin and Maurice, 2008).

Table 5. Effect of treatments on the moisture content (wet basis) of basil leaves (n = 3)

Treatment	% MC	Water activity
Control (Fresh)	91.20±0.10C	0.92±0.06A
ABA	92.53±0.12B	0.52±0.02B
FC	92.50±0.10B	0.52±0.01B
Survived, opened stomata electroporated	92.17±0.06B	0.53±0.03B
Survived, opened stomata not electroporated	92.60±0.10B	0.51±0.02B
Fully disintegrated cells	92.87±0.06B	0.53±0.02B
VI + Survived, opened stomata electroporated	93.23±0.001A	0.51±0.03B
VI + Control	93.09±0.001B	0.52±0.01B

Means ± SD followed by different letters within a column are significantly different (P < 0.05)

5.2. Effect of ABA and FC on stomatal movement and drying curves

Figure 2 and Table 6 show the response of stomatal aperture to ABA and FC concentrations. After exposure to 1 h white light, the stomata of the untreated samples were opened to approximately 16.72 ± 1.30 μm (Figure 2A). Incubating the leaves in 10 μM of ABA

solution for 2 h induced complete closure of the stomata (Figure 2B). Whilst the stomata aperture was totally closed under the ABA treatment, it was opened in the FC treatment (Figure 2C). Incubating leaves in 5 µM FC solution significantly ($P < 0.05$) increased stomatal aperture from the initial 16.72 ± 1.30 µm opening induced by white light to 47.57 ± 2.82 µm, representing 185% increase in stomatal aperture after 2 h of incubation.

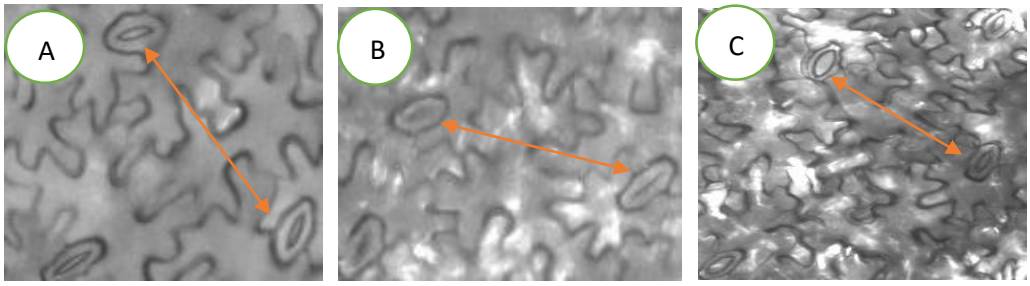

Figure 2. Microscopic view of *Ocimum basilicum L.* Stomata taking after plants were exposed to white light for 1 h (A) before incubating leaves in white light under treatment with either (B) 10 µM ABA or 5 µM FC solutions for 2 h. Images were taking at a magnification of 20X. The arrows in the figure are pointing to the stomata.

Table 6. Effect of ABA and FC on stomatal aperture, measurements at 20X (n = 20).

Treatment	Stomatal aperture (µm)
Control	16.72±1.30B
ABA	-
FC	47.57±2.82A

Means ± SD followed by different letters within a column are significantly different at ($P < 0.05$).

Figure 3 shows the drying curves for leaf samples treated with the chemical solutions. The samples were dried from an initial moisture content of 10.36, 12.39 and 12.33 kg water per kg dry weight for the control, ABA-treated and FC-treated samples respectively. The drying curves show that stomatal movement has significant effect on the drying kinetics. Complete stomatal closure induced by ABA resulted in a slow moisture removal whereas increased stomatal aperture induced by FC resulted in a rapid drying of the leaves compared to the control.

The dimensionless moisture ratio decreased continuously with drying time till the end of drying. However, the loss of water from the leaves in all treatments and the control was rapid during the first 100 min after which the loss of water decreases and slowly flattened out toward the end of drying. However, there were differences in the variation of moisture ratio with time. After 100 min of drying, the dimensionless moisture ratio of the control has reduced from the initial 1.0 to 0.33. The dimensionless moisture ratio of samples treated with FC had reduced from the initial 1.0 to 0.20 whilst that of samples treated with ABA had

decreased from 1.00 to 0.43. These losses represent 63%, 60% and 81% loss of moisture for the control, ABA-treated and FC-treated samples respectively.

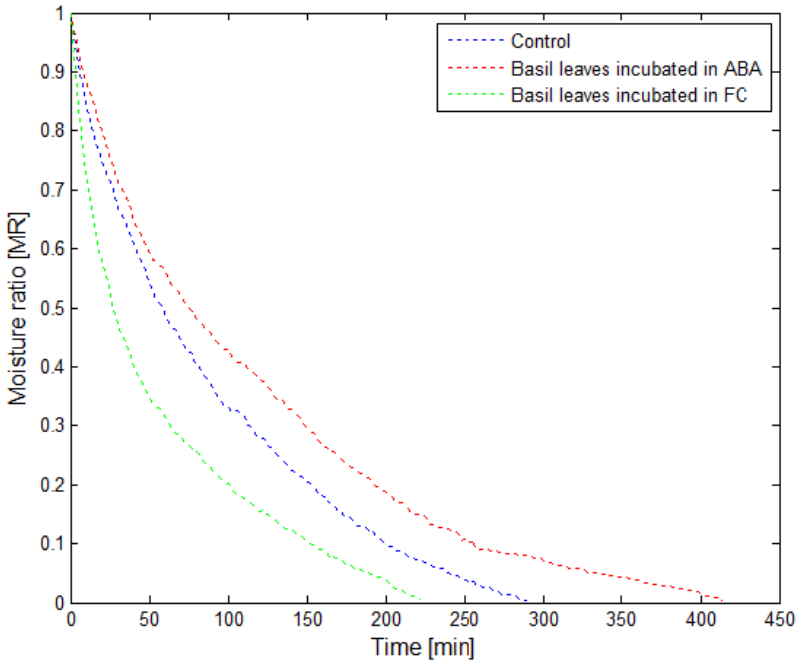

Figure 3. Effect of ABA and FC on the convective drying curve of basil leaves samples. Samples were exposed to light for 1 h (blue line) (stomata aperture 16.72 µm) and later incubated in either 10 µM ABA solution (red line) (stomata aperture 0 µm) or 5 µM FC solution (green line) (stomata aperture 47.57 µm) for 2 h prior to drying in a convective oven at 50 ℃ and 2 m/s air rate. Data points are average of two replications which do not differ from each other by more than 4%.

5.3. Effect of PEF-treatment on the drying curves

5.3.1. Effect of PEF on stomata

All leaves which have taken up PI in their nuclei after electroporation are assumed to be permeabilized in response to the electric pulses. There was uniform electroporation under the studied electric conditions (Figure 4A) but different combinations of the pulsed electric parameters influenced stomatal electroporation (Figure 4B and 4C).

When the leaves were electroporated at a field strength of 0.6 KV/cm, pulse width of 120 µs, pulse space of 500 µs and 65 pulses, there was uniform electroporation of the epidermal cells (Figure 4A) but the stomata were not electroporated (Figure 4C). The result shows that at the same field strength and number of pulses (as in Figure 4A), increasing the pulse width to 150 µs and the pulse space to 760 µs resulted in the opened stomata been electroporated (Figure 4B).

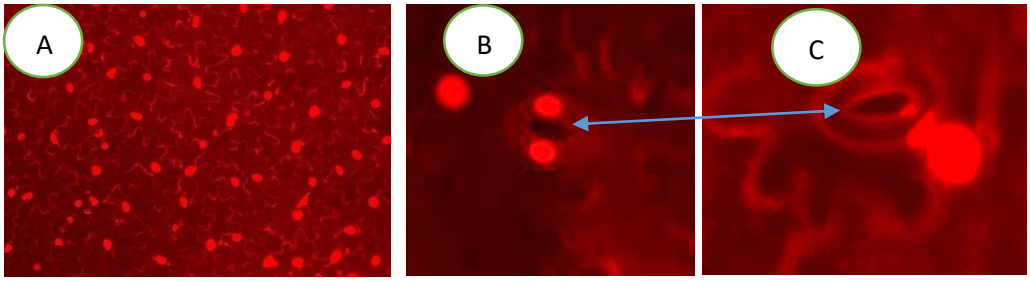

Figure 4. Effect of electric parameters on electroporation conditions. Microscopic view of *Ocimum basilicum L*. cells taking after plants were exposed to light for 1 h before reversible permeabilization in 250 µM PI. (A) shows uniform electroporation of epidermal cells; (B) the opened stomata are electroporated at PEF conditions of field strengths of 0.6 KV/cm, pulse width of 150 µs, pulse space of 760 µs and 65 pulses; (C) the opened stomata are not electroporated at PEF conditions of field strength of 0.6 KV/cm, pulse width of 120 µs, pulse space of 500 µs and 65 pulses. The arrow in B and C points to the stomata. A was taken at 10X whilst B and C were taken at 20X magnification.

5.3.2. Effect of PEF parameters on cell viability

Figure 5 shows the microscopic view of the cells monitored by FDA staining 20 h after electroporation. There was no loss in viability of cells, shown by the complete FDA staining of cells electroporated at 0.6 KV/cm field strength (Figures 5B and 5C) and the control fresh basil leaves, non-electroporated (Figure 5A). There was complete loss of viability in the cells electroporated at 1.5 KV/cm filed strength (Figure 5D) and the negative-non electroporated but killed in the freezer (5E). The loss in viability shown in Figure 5D is a result of irreversible cell damage inflicted by the high field strength treatment.

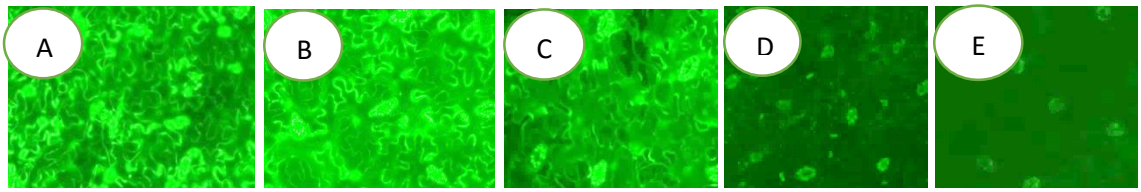

Figure 5. Test of cell viability after 20 h of electroporation. Microscopic view of *Ocimum basilicum L*. cells taking after leaves were incubated in 12 µM FDA for 1 h. (A) is the positive control (fresh basil leaves). PEF-treated samples were stored at 4 ℃ for 20 h before incubation. The PEF parameters for (B) are field strength of 0.6 KV/cm, pulse width of 120 µs, pulse space of 0.5 ms and 65 pulses). (C) and (D) were electroporated at field strengths of 0.6 KV/cm and 1.5 KV/cm respectively with other PEF parameters (pulse width of 150 µs, pulse space of 0.76 ms and 65 pulses) constant (E) is the negative control (dead cells, leaves were stored in the freezer for 10 min before incubation). Images were taking at a magnification of 10X.

5.3.3. Drying curves

Figure 6 shows the plot of moisture ratio (M_R) vs time (t) obtained from experimental data for the PEF treated samples. The samples were dried from an initial moisture ratio of 10.36, 11.83, 12.51 and 12.86 kg water per kg dry weight respectively for:

- The control,
- Reversible electroporation of epidermal cells with opened, electroporated stomata
- Reversible electroporation of epidermal cells with not electroporated, opened stomata (after light exposure),
- Irreversible electroporation of epidermal cells and opened stomata after light exposure

The loss of water from the leaves changed rapidly during the first 100 min after which the loss of water decreases and slowly flattened out toward the end of drying. However, PEF conditions affected the variation of moisture ratio with time. The initial dimensionless moisture ratios had respectively fallen to 0.33, 0.16, 0.10 and 0.04 for the control, reversible electroporation of epidermal cells with opened electroporated stomata, reversible electroporation of epidermal cells with opened stomata not electroporated, and irreversible electroporation of epidermal cells with opened stomata after 100 min of drying.

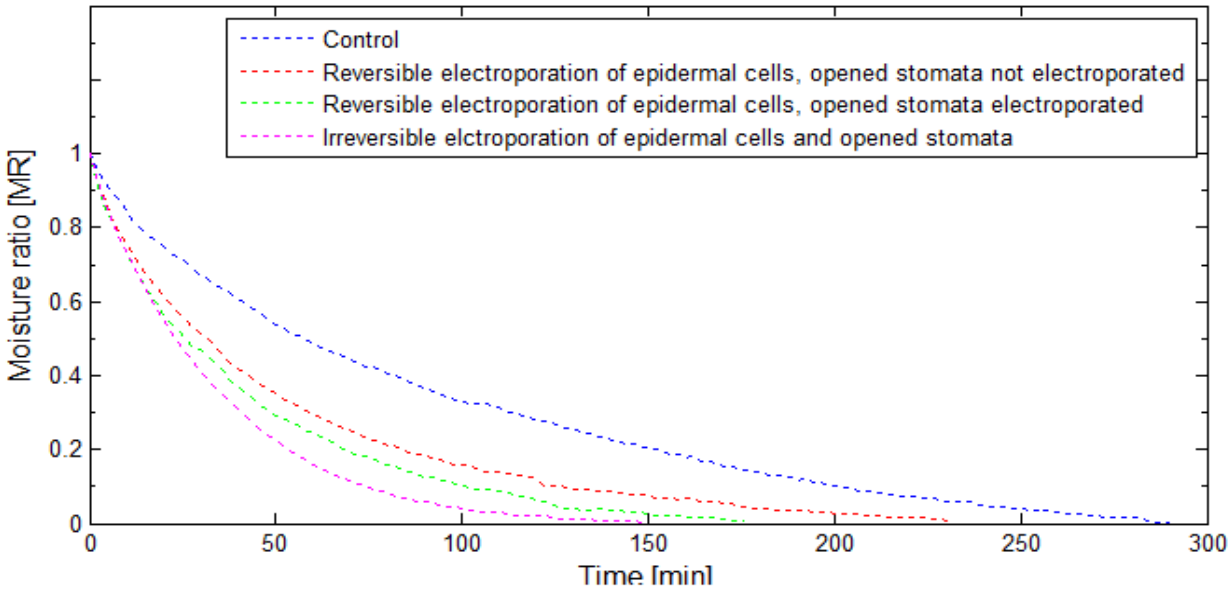

Figure 6. Effect of PEF parameters on the convective air drying of basil leaves. Convective drying of the samples was carried out 50 ℃ and 2 m/s air velocity. Both samples were exposed to light for 1 h and either immediately dried (blue line) or PEF-treated before drying. PEF parameters are (red line)- field strength of 0.6 KV/cm, pulse width of 120 µs, pulse space of 0.5 ms and 65 pulses; (green line) - field strength of 0.6 KV/cm, pulse width of 150 µs, pulse space of 0.76 ms and 65 pulses and (violet line) - field strength of 1.5 KV/cm, pulse width of 150 µs, pulse space of 0.76 ms and 65 pulses. Data points represent the average of two replications with maximum of 4.0 % deviation from each other depending on the stages of drying and the treatment.

Whilst both PEF-treated samples dried faster compared to the control, the rate of drying was fast at higher field strength where the cells had completely been damaged throughout the drying process. On the samples treated at the same field strength, where there was reversible electroporation, the drying rate was relatively parallel for the first 100 min of drying beyond

which samples treated with PEF conditions optimized for the opened stomata to be electroporated dried significantly faster than samples in which the opened stomata is not electroporated. If stomata closes during drying was not monitored during this study.

Samples vacuum impregnated with trehalose solution dried from the initial moisture content of 13.47 and 13.77 kg water per kg dry weight respectively for samples that were

- only vacuum impregnated and
- vacuum impregnated and PEF-treated.

VI with trehalose solution resulted in greater rate of moisture removal compared to the control non-impregnated samples (Figure 7). However, the combined treatment of vacuum infusion and PEF application did not improve the rate of moisture removal compared to samples that were only PEF-treated with opened stomata electroporated.

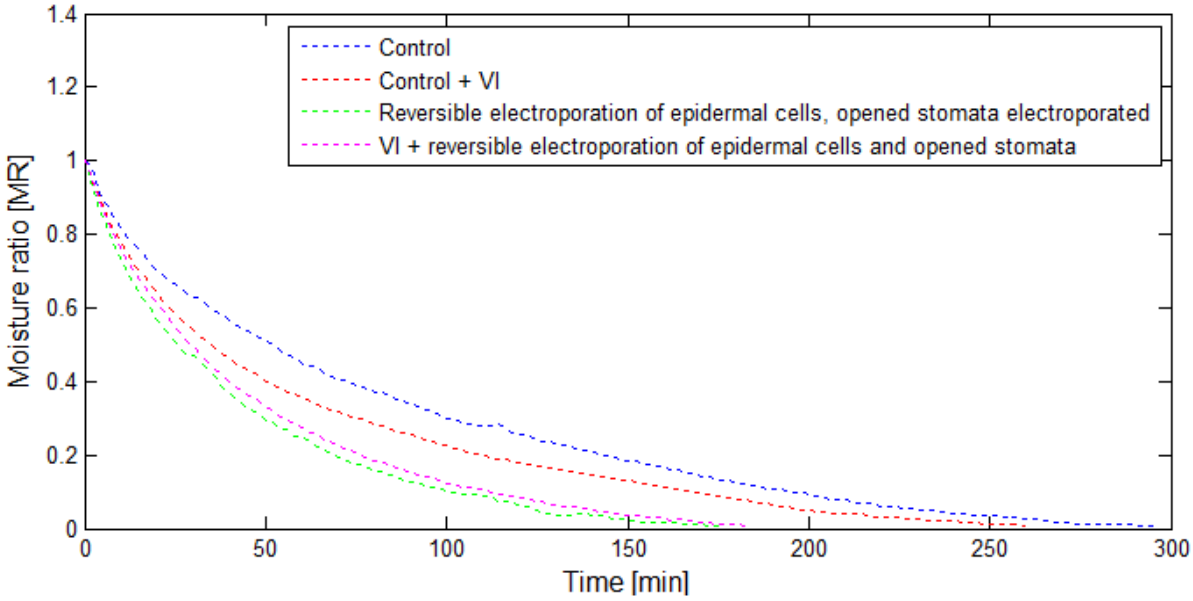

Figure 7. Combined effect of vacuum infusion with trehalose and PEF treatment on the convective air drying of basil leaves. Convective air drying of the samples was carried out 50 ℃ and air velocity of 2 m/s. both samples were exposed to light for 1 h and either immediately dried (blue line) or vacuum impregnated with trehalose before drying (red line). green line was only PEF-treated at field parameters: field strength of 0.6 KV/cm, pulse width of 150 µs, pulse space of 0.76 ms and 65 pulses whilst (violet line) is a combination of vacuum impregnation with trehalose and PEF-treatment- with same PEF parameters as in green line. Data points are average of two replications with an average deviation from each other in the range of 0.0 to 4.0 % depending on the stages of drying and the treatment.

5.4. Mathematical modelling of the drying curves

Figures 8-10 and Table 7 show the variation of drying rate with dimensionless moisture ratio for the various treatments. The results show that the moisture content has a significant effect on the drying rate. Higher drying rates were observed at the beginning of drying and the

drying rate decreased continuously with both moisture ratio and drying time. This means that there was no constant rate period and the entire drying process occurs in the falling rate period. Fitting the Newton model to the drying curves resulted in drying rate at the initial stages of drying in the range of 0.0088 to 0.25 min^{-1} and the drying rate towards the later stages of drying ranges from 0.00024 to 0.00039 min^{-1} (Table 7). All the treatments except the 2 h incubation in ABA resulted in higher initial drying rate compare to the control. Among the PEF-treated samples, higher initial drying rate (0.03 min^{-1}) was observed in leaf samples subjected to irreversible permeabilization at higher field strength compared to samples subjected to reversible permeabilization at low field strength (0.0202 to 0.025 min^{-1}).

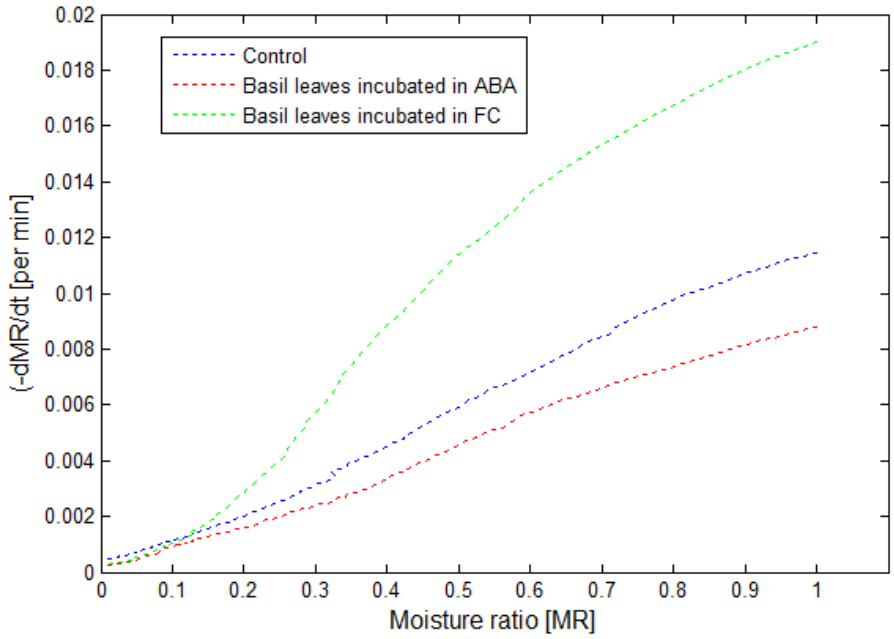

Figure 8. Variation of drying rate with moisture ratio for chemically treated samples. Treatment and drying conditions are the same for each treatment as described in the *MR* vs time curves in Fig. 3.

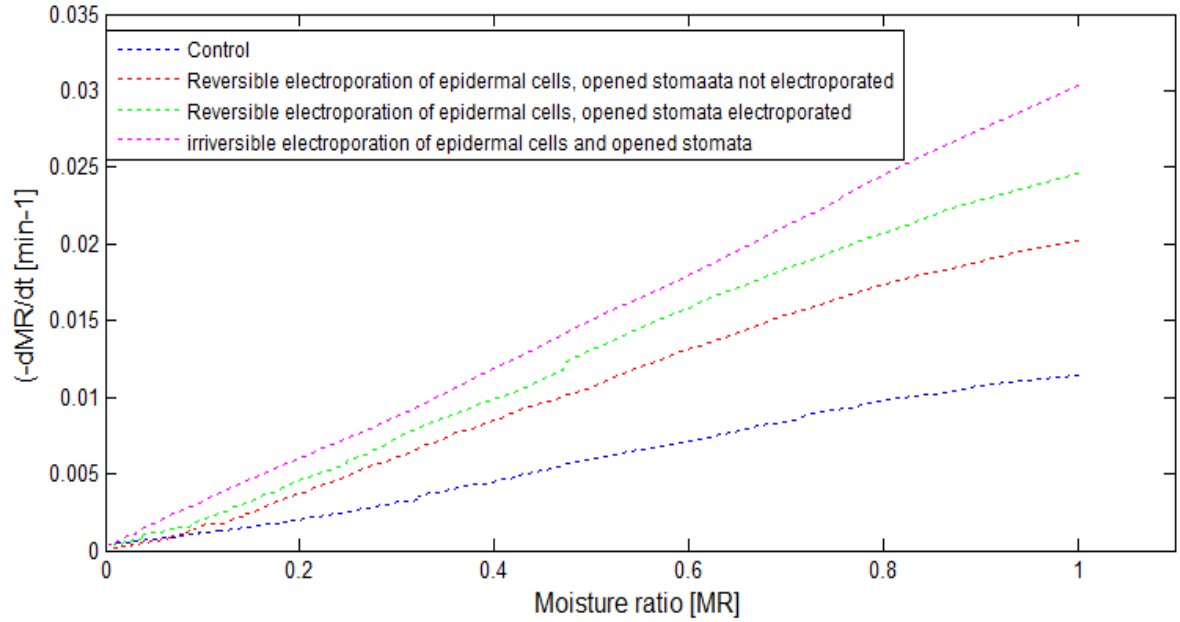

Figure 9. Variation of drying rate with moisture ratio for PEF treated samples. Treatment and drying conditions are the same for each treatment as described in the *MR* vs time curves in Figure 6.

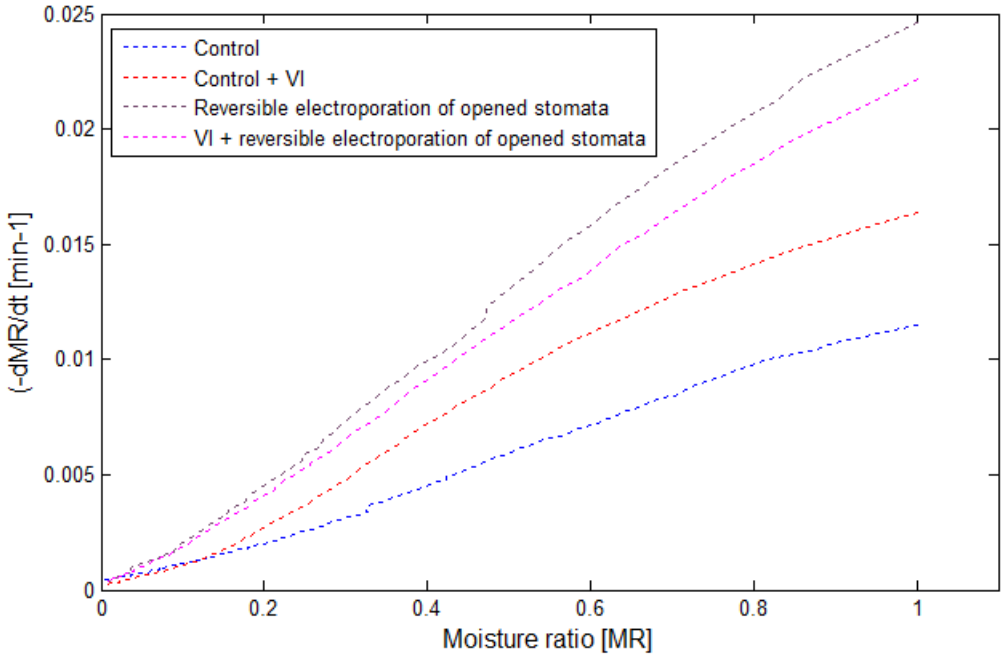

Figure 10. Variation of drying rate with moisture ratio for vacuum impregnated samples. Treatment and drying conditions are the same for each treatment as described in the *MR* vs time curves in Fig. 7.

Among samples with reversible electroporation at low field strength, reversible electroporation of opened electroporated stomata resulted in faster drying rate (from 0.025 to 0.00039) compared to samples with opened stomata not electroporated (from 0.0202 to 0.0021) throughout the drying period. Vacuum impregnation with trehalose increased the initial drying rate of the control from 0.012 to 0.016 min^{-1} (Table 7) but towards the end of drying, the drying rate of the control and vacuum infused control were slightly the same

(0.0025 and 0.0024 min^{-1}) respectively. Combination of vacuum infusion and PEF treatment however decreased the drying rate compared to the drying rate of samples that were only PEF-treated. Vacuum infusion before PEF treatment decreased the initial drying rate of the PEF-treated samples from 0.025 to 0.022 min^{-1}.

Table 7. Effective moisture diffusivity and drying rate obtained for selected moisture ratios.

Treatment	D_{eff} **($m^2 s^{-1}$)**	**Drying rate (min^{-1})**		
		MR = 1	MR = 0.5	MR = 0.01
Control	$1.036*10^{-9}$	0.0120	0.0060	0.0005
ABA	$7.076*10^{-10}$	0.0088	0.0050	0.0003
FC	$1.204*10^{-9}$	0.0190	0.0120	0.0030
Survived, opened stomata electroporated	$1.857*10^{-9}$	0.0250	0.0120	0.0004
Survived, opened stomata not electroporated	$1.309*10^{-9}$	0.0202	0.0110	0.0002
Fully disintegrated cells	$2.586*10^{-9}$	0.0300	0.0160	0.0052
VI + Survived, opened stomata electroporated	$1.171*10^{-9}$	0.0220	0.0120	0.0004
VI + Control	$1.718*10^{-9}$	0.016	0.0091	0.0002

The fit parameters obtained after fitting the Newton model to the experimental drying curves are given in Table 8. A good fit of experimental data was observed with all the calculated R^2 values within the range of 0.999-0.942 and the observed RMSE, which is a measure of the deviation of the fitted curve from the experimental data within the range of 0.014 to 0.053. The closer the RMSE value to zero, the more accurate the curve is for predictability.

Table 8. Statistical results obtained after fitting the Newton model to the drying curves to determine the drying rates.

Treatment	**R^2**	**SSE**	**RMSE**
Control	0.985	0.129	0.030
ABA	0.992	0.105	0.023
FC	0.942	0.307	0.053
Survived, opened stomata electroporated	0.997	0.018	0.014
Survived, opened stomata not electroporated	0.987	0.077	0.026
Fully disintegrated cells	0.999	0.002	0.005
VI+ Survived, opened stomata electroporated	0.996	0.022	0.015
VI + Control	0.974	0.181	0.037

The drying curves and the drying rate vs moisture ratio curves show that all the drying occurred in the falling rate period. The diffusion model was used to evaluate the effects of the treatments on mass transport within the leaf tissue at the falling rate period. Linear regression analysis of the drying curves shows concave moisture diffusivity curves (not shown). The obtained effective moisture diffusivity values were in the range $7.076*10^{-10}$ to $2.586*10^{-9}$ m^2 s^{-1} (Table 7). Apart from chemical incubation with ABA which decreased the effective moisture diffusivity, all the other treatments increased the effective moisture diffusivity compared to the control. Stomatal electroporation also resulted in increased effective moisture diffusivity ($1.857*10^{-9}$ m^2 s^{-1}) compared to samples where the opened stomata were not electroporated ($1.309*10^{-9}$ m^2 s^{-1}). The effective moisture diffusivity values of basil leaves obtained for each treatment in this studies are within the range of 10^{-7} to 10^{-11} m^2 s^{-1} reported for biological materials (Wang et al., 2007) but were however higher than the values $6.44*10^{-12}$ m^2 s^{-1} (Akpinar, 2006) reported for basil. Differences in the effective moisture diffusivity values may be attributed to the treatments applied and the drying medium used.

Table 9. Experimentally determined drying times to dry to 10 % moisture content (dry basil) and the dry weight after drying basil leaf samples to the predicted drying time.

Treatment	drying time (min)	% dry weight
Control	279	89.98 ± 0.02
ABA	406	-
FC	217	-
Survived, Stomata opened and electroporated	177	90.03 ± 0.03
Survived, Stomata opened but not electroporated	228	89.99 ± 0.02
Fully disintegrated cells	148	90.05 ± 0.01
Control + VI	248	90.10 ± 0.01
VI + Survived, opened stomata electroporated	181	90.25 ± 0.02

The increased mass transport and the resultant increase in the drying rates due to the applied treatments (except the sample incubated with ABA), resulted in reduced drying time needed to reach an expected moisture ratio compared with the control. The experimentally determined drying times needed to reach moisture content of 10% (kg water per kg dry weight) are summarised in Table 9. Compared to the control, irreversible electroporation of the leaf tissue resulted in 47% reduction whilst reversible electroporation of the epidermal cells with opened stomata electroporated resulted in 37% reduction in the convective air drying time of basil leaves. Reversible electroporation of the epidermal cells but with opened stomata not electroporated could only result in 18% reduction in the experimentally

determined drying time. Vacuum infusion with trehalose also resulted in 11% reduction in the convective air drying time compared to the control. The combination of vacuum infusion with trehalose and PEF-treatment did not have any influence on the final drying time compared to PEF treatment alone.

5.5. Effect of treatments on colour change

The colour evaluation parameters are given in Table 10. The colour brightness (L*), greenness (negative a*) and yellowness (positive b*) for the fresh basil leaves are 35.66±3.71, -9.45±0.32 and 21.02± 2.92 respectively. Pre-drying and drying operations altered the colour of the dried leaf samples compared to the fresh basil leaf. The colour brightness of the untreated dried samples and samples that were vacuum impregnated with trehalose solution prior to drying were significantly ($P < 0.05$) lower when compared to either the fresh leaves or the untreated dried samples. There was no change in colour brightness between the fresh leaves and PEF-treated samples. The colour greenness significantly ($P < 0.05$) decreased with pre-drying treatment and with drying compared to the fresh leaves. Samples that were pre-treated before drying recorded significantly ($P < 0.05$) higher values for the colour greenness compared to the untreated dried samples. Vacuum impregnation with trehalose solution resulted in intense greener colour prior to drying but drying of the vacuum impregnated samples significantly ($P < 0.05$) decreased the colour greenness compared to the fresh leaves and the untreated dried samples. The colour yellowness significantly ($P < 0.05$) decreased in the untreated dried samples and dried samples that were either irreversibly electroporated of vacuum impregnated with trehalose prior to drying when compared to the fresh leaves.

Table 10. Effect of treatments on colour change of dried basil leave samples (n = 20)

Treatment	L*	a*	b*	ΔE
Control (fresh)	35.66±3.71A	-9.45 ±0.32A	21.02± 2.92A	
Control (dried)	32.65 ± 65BCD	-2.16 ± 1.09 F	17.13 ± 2.20B	**8.91**
Opened stomata not reversible electroporated	34.12 ± 3.30AB	-5.58 ± 0.46C	19.62 ± 2.00A	**4.40**
Reversible electroporation of opened stomata and epidermal cells	34.73 ± 3.28AB	-6.90 ± 0.28B	20.94 ± 2.14A	**2.72**
Irreversible electroporation of epidermal cells	33.40 ± 2.64ABC	-3.31 ± 0.64 E	16.99 ± 1.90B	**7.79**
Control + VI	30.05 ± 1.35 CD	-4.60 ± 0.30D	16.21 ± 2.37B	**8.83**
VI + reversible electroporation of opened stomata	30.91 ± 3.32D	-3.46 ± 0.89E	13.36 ± 2.49C	**10.82**

Means ± SD followed by different letters within a column are significantly different ($P < 0.05$)

The total colour change values (ΔE) for the dried leaf samples were in the range 2.72 to 10.82. Again, samples PEF-treated before drying produced less total colour change compared whilst samples vacuum impregnated with trehalose or combination of VI and PEF-treatment before drying resulted in higher total colour change. Between the PEF-treated samples, opened electroporated stomata resulted in less total colour change compared to dried samples in which opened stomata were not electroporated. Adopting the descriptive level of colour change used by Chen (2008), the obtained total colour change values ranges from noticeable difference for samples with opened electroporated stomata prior to drying to large difference for samples vacuum impregnated with trehalose solution before drying.

5.6. Effect of the treatments on rehydration properties

Figure 11 and Table 11 respectively show a graph of rehydration ratio with time and the percent weight recovered after rehydrating to constant weight. All the pre-drying treatments resulted in better rehydration ratio compared to the control (A).

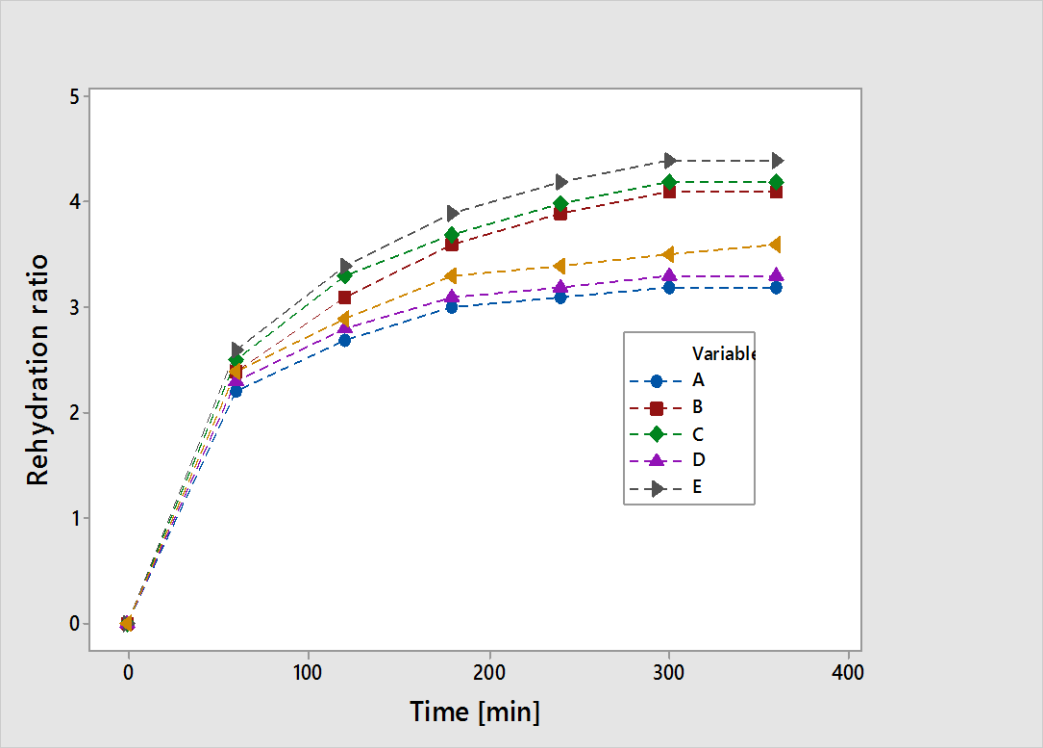

Figure 11. Variation of rehydration ratio with time. The following treatments: (A):- the control (untreated), (B):- reversibly electroporation with opened stomata not electroporated, (C):- reversible electroporation of opened stomata, (D):- irreversible electroporation of epidermal cells, (E):- vacuum impregnation with trehalose before PEF-treatment with PEF parameters as in D, (F):- vacuum impregnation of the control with trehalose was applied prior to drying. Data points are averages of three replications.

Among PEF-treated samples, samples that were reversibly electroporated (B and C) significantly ($P < 0.05$) recovered more of the weight lost during dehydration compared to samples that were irreversibly electroporated (D) prior to drying. The combination of vacuum infusion with trehalose and PEF-treatment (E) recorded the highest rehydration ratio whist samples that were only vacuum infused with trehalose (F) before drying recorded slightly better rehydration properties compared to the control non-treated samples (A).

Table 11. Weight recovery after rehydrating to constant weight at room temperature.

Treatment[1]	Weight of fresh leaves	Weight after rehydrating to constant weight	% of weight recovered after rehydrating to constant weight
A	2.05±0.08	1.12±0.01E	53.63±0.01E
B	2.05±0.08	1.46±0.02B	71.22±0.02B
C	2.05±0.08	1.48±0.00B	72.20±0.00B
D	2.05±0.08	1.17±0.01D	57.07±0.01D
E	2.05±0.08	1.57±0.02A	76.59±0.02A
F	2.05±0.08	1.25±0.02C	60.98±0.02C

[1] Treatments are: (A) The control (untreated), (B):- reversibly electroporation with opened stomata not electroporated, (C):- reversible electroporation of opened stomata, (D):- irreversible electroporation of epidermal cells, (E):- vacuum impregnation with trehalose before PEF-treatment with PEF parameters as in C, (F):- vacuum impregnation of the control with trehalose was applied prior to drying. Reported are average data of three replications. Means ± SD followed by different letters within a column are significantly different ($P < 0.05$).

5.7. Effect of treatments on composition of chemical compounds

Table 12 shows the GC-MS aroma profile and Figure 12 shows a typical chromatogram of aroma compounds of Genovese basil extracted by supercritical fluid CO_2 expanded ethanol. Nineteen aroma compounds were identified and the GC-MS spectral shows the main aroma compounds from Genovese basil cultivated in Sweden are methyleugenol (31.7%), caryyophellene oxide (9.12%), eugenol (8.04%) and cis-beta-farnesene (5.09%). Other sesquiterpene hydrocarbons (such as cubebene, bergamotene), oxygenated sesquiterpene (farnesol), monoterpene hydrocarbons (beta-ocimene) and oxygenated monoterpene (menthol) were in substantial amount.

The pre-drying treatments and drying of the leaf samples produced changes in the aroma profile. All the major identified aroma compounds in the fresh leaves were present in the dried samples whilst some minor aroma compounds were below detection limit in some pre-treated dried samples. The concentrations of three aroma compounds; bergamotene, beta-ocimene and menthol in all PEF-treated dried samples were below detection limit. The concentrations of carvacrol, cubebene and cadinol were also below detection limits in all

dried samples. Pinene oxide was either below detection limit or unseparated from caryophellene oxide in all dried samples.

Table 12. Chemical compounds from fresh Genovese basil leaves, extracted by supercritical fluid CO_2 expanded ethanol and analysed by GC-MS

Peak	Retention time [min]	Compounds
1	11.8	Carvacrol[a]
2	13.3	Eugenol
3	14.9	Methyleugenol
4	15.9	Bergamotene[b]
5	16.3	beta –ocimene[b]
6	16.7	Cis-beta farnesene
7	17.3	Cubebene[a]
8	21.2	Dodecanol
9	22.4	Cadinol[a]
10	28.4	Octadecene
11	28.6	Cis-9-tetradecen-1-ol
12	29.3	Cytronellyl butyrate
13	29.8	Menthol
14	32.9	Ethyl palmitate
15	35.9	Phytol
16	37.0	Pinene oxide[a]
17	37.3	Caryophellene oxide
18	40.3	Hexadecanoic acid[a]
19	51.3	Farnesol

[a] concentration below detection limit in all dried samples

[b] concentration below detection limit in all samples PEF-treated before drying

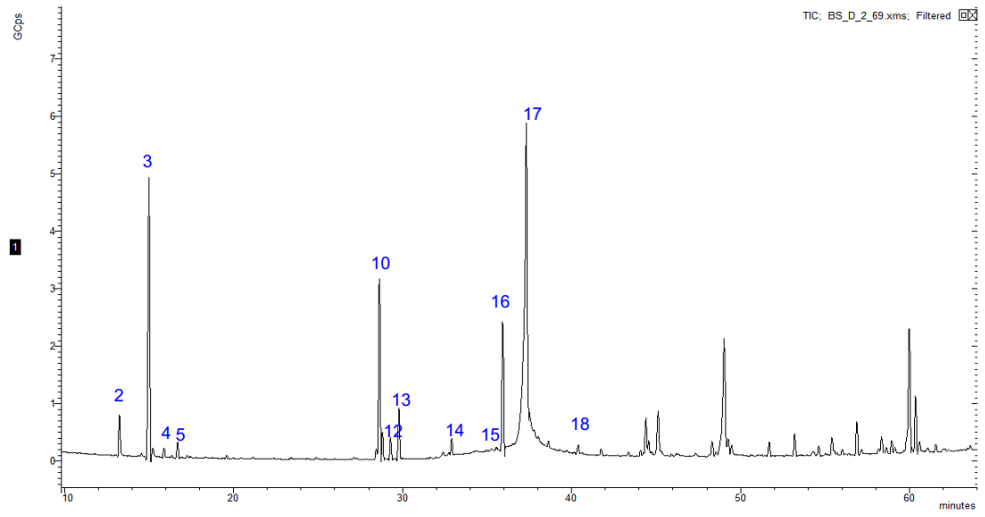

Figure 12. Typical chromatogram of chemical compounds extracted from Genovese basil by direct injection in GC-MS. The peak numbers correspond to the numbers in Table 12.

Table 13. Relative increase in aroma compounds of Genovese basil in the treated dried samples compared to the untreated dried sample.

Compounds	**A**	**B**	**C**	**D**	**E**
Eugenol	1.77±0.02B	2.32±0.06A	1.81±0.05B	0.54±0.02D	0.83±0.04C
Methyl eugenol	1.19±0.05A	1.31±0.09A	0.98±0.05B	0.47±0.03C	0.49±0.04C
Cis-beta-farnesene	1.11±0.11B	2.07±0.11A	0.76±0.06C	0.43±0.03D	0.47±0.03D
dodecanol	1.12±0.01B	1.45±0.01A	0.73±0.02C	0.31±0.01D	0.32±0.02D
Octadecene	1.30±0.03B	1.92±0.11A	1.15±0.03C	0.28±0.01D	0.36±0.01D
Cis-9-tetradecen-1-ol	1.29±0.02B	1.65±0.12A	1.33±0.09B	0.18±0.01D	0.41±0.02C
Menthol	1.32±0.06B	1.65±0.08A	1.30±0.06B	0.17±0.01C	0.15±0.01C
Ethyl pamitate	1.21±0.04B	1.30±0.04B	1.65±0.06A	0.94±0.04C	0.96±0.04C
phytol	1.15±0.06C	1.56±0.05A	1.29±0.06B	0.29±0.00E	0.61±0.01D
Caryophellene oxide	3.14±0.30B	5.74±0.23A	1.37±0.10C	0.61±0.02D	0.76±0.02D
farnesol	0.98±0.04D	1.42±0.06B	1.17±0.06C	1.64±0.06A	1.06±0.06CD

The following treatments: (A):- reversibly electroporation with opened stomata not electroporated, (B):- reversible electroporation of opened stomata, (C):- irreversible electroporation of epidermal cells, (D):- vacuum impregnation with trehalose before PEF-treatment with same PEF parameters as that of B, (E):- vacuum impregnation of the control with trehalose was applied prior to drying. Data points are average of three replications. Means of three replicates ± SD followed by different letters within a row are significantly different ($P < 0.05$)

Eleven of the identified aroma compounds in the fresh leaves were fully separated and identified in all dried samples. These eleven aroma compounds were chosen to evaluate the effects of the pre-drying treatments on the concentration of aroma compounds relative to the untreated dried sample (Table 13). The abundance of these eleven compounds were higher in all the treated dried samples relative to the untreated dried sample. The reduced drying times as a result of PEF-treatment resulted in high abundance of the aroma compounds relative to the untreated dried sample. The four major aroma compounds; methyleugenol, caryophellene oxide, eugenol and cis=beta-farnesene identified in the fresh leaves were:

1. 0.76 to 1.37 fold higher in dried samples that were irreversibly electroporated prior to drying compared to untreated dried samples;
2. 1.19 to 3.14 fold higher in dried samples that were reversibly electroporated but with opened stomata not electroporated prior to drying compared to untreated dried samples.
3. 1.31 to 5.74 fold higher in dried samples that were reversibly electroporated with the opened stomata electroporated prior to drying compared to untreated dried samples.

The major volatiles identified in the treated dried samples were significantly ($P < 0.05$) higher in dried samples in which stomata was electroporated. The combination of vacuum impregnation with trehalose and reversible electroporation of opened stomata however significantly ($P < 0.05$) decreased the abundance of the aroma compounds compared to the samples that were only reversible electroporated with the opened stomata electroporated.

6. Discussion

6.1. Stomatal movement

In the present study, stomatal opening in the presence of light was observed after treating leaf samples in 5 μM FC solution. Exogenous application of 10 μM ABA to light-induced opened stomata promoted complete stomatal closure after 2 h of treatment. The regulation of plasma membrane H^+ ATPase and K^+ ions and other osmolytes are responsible for the observed opposite effects of ABA and FC on stomatal movement. Light induced stomatal movement is known to depend on the osmotic regulation of K^+ ions and other solutes such as chloride and sugars. Assmann and Schwartz (1992) observed a synergistic effect of light and low FC concentrations on stomatal opening of epidermal peel of Asiatic Dayflower (*Commelina communis)* and broad bean (*Vicia faba).*

The effect of FC on stomatal opening is attributed to its ability to activate plasma membrane H^+ ATPase (Assmann and Schwartz, 1992; Braunsgaard et al., 1998). ABA has been implicated in both light and dark-induced stomatal closure and its action in regulating light-induced stomata closure is believed to occur through signalling components such as Ca^{2+} (Kwak et al., 2003) which serves as an intermediate in the stomatal closing cascade induced by ABA. These results further confirm the opposite effects of ABA and FC on stomatal movement.

6.2. Electroporation and cell viability

PI cell staining has been used in several studies to study the electropermeabilization of cell membranes (Joersbo et al, 1990; Gómez Galindo et al., 2008). Since PI cannot enter intact plant cell membranes, the presence of red colour of PI staining in the nuclei is indicative of cell membrane permeabilization in response to electrical pulses. The PI staining results show cells with either the stomata electroporated or not in addition to uniform electroporation of the epidermal cells. Stomatal electroporation is dependent on the combination of the electrical parameters. Our results show that optimization of the pulse width and pulse space are necessary to achieve stomata electroporation. For basil, preliminary trials (results not given in this report) shows a narrow range of pulse width (140 - 160 μs) and pulse space (750 - 760 μs) as optimal conditions to achieve stomatal electroporation and these optimal conditions must be combined to achieve full stomata electroporation.

FDA staining is often used to differentiate between reversibly damaged and irreversibly damaged electropermeabilized cell membranes (Dymek et al., 2014a). The FDA staining assay gives a distinction between dead and living cells. The study was not designed to determine the threshold field strength to recover cell viability in basil leaves after electroporation but the FDA assay shows that if the applied field strength is low enough, in our case 0.6 KV/cm (probably below the threshold value for basil cell membrane), the electroporation is reversible and the viability of cells is not affected. $H^{`+}$-ATPase is the main trans-membrane transport channel in living cells. It is generally accepted that the activity of $H^{`+}$-ATPase helps the cell to take up ions leaked from partially damaged cell membranes (Arora and Palta, 1991). The complete loss of viability at higher field strength (1.5 KV/cm) is indicative of electrical parameters beyond the threshold values. The ions in a cell move to the membrane surface in response to an external electric field. The electric pulses create pores in the cell membrane and the cell membrane breaks down when the transmembrane potential reaches a threshold value (Hibino et al., 1991).

6.3. Effect of treatments on drying kinetics

The experimental drying curves confirm that stomatal aperture has significant effect on the drying rate and the mass transfer within the drying basil leaves (see Table 9). In describing the drying kinetics of treated basil samples, attention is given to the determination of the drying rate and the effective moisture diffusivity. Samples treated with 10 µM ABA solution, and in which the stomata were microscopically confirmed to be closed, shows significantly reduced rate of moisture removal, thereby elongating the drying rate and the drying time by 127 min compared to the control. However, samples treated with FC dried significantly faster (217 min). This result demonstrates that stomata aperture has significant effect on drying kinetics of detached plant parts and was further confirmed in the PEF treated samples.

For the PEF treated samples, samples treated at higher field strength (1.5 KV/cm) resulted in faster drying (148 min to reach moisture ratio of 0.1 kg water per kg dry weight) whilst samples treated at lower field strength (0.6 KV/cm) were dried after 177 and 228 min, for stomata opened electroporated and opened stomata not electroporated, respectively. These results suggest that the irreversible membrane damage as a result of the higher field strength (1.5 KV/cm) treatment significantly reduced the drying time by 131 min compared to the control but could only reduce the drying time by 29 min when compared to the drying time of samples treated at low field strength provoking reversible electroporation of opened stomata

(177 min). This proves that the drying time of samples can significantly be reduced without necessary disintegrating the cell membrane but only by affecting the stomata with electroporation.

Among the samples which survived electroporation, electroporation of opened stomata resulted in 51 min reduction in the drying time compared to samples in which opened stomata electroporation was not achieved. Since the samples with and without opened stomatal electroporation were treated at the same field strength (the main electrical parameter which influences reversibility after electroporation), the difference in their drying times could be attributed to the electroporated opened stomata. The electroporated opened stomata might have remained opened for most of the drying period thereby enhancing the rate of moisture removal during drying.

The difference in drying between samples treated at higher field (1.5 KV/cm) and samples treated at lower field (0.6 KV/cm) was more obvious after just 25 min of drying. The diffusion of water from the interior of the leaf tissues to the drying surface controls the rate of drying (Wang et al., 2007). The mechanism of mass transfer in biological materials is very complex but in the falling rate period, the drying rate is assumed to be controlled by the diffusion of moisture from the interior of the leave tissue to the drying surface. The term effective moisture diffusivity is used to encompass all the known and unknown diffusional processes occurring at the falling rate period. Moisture diffusivity is affected by composition, moisture content and porosity of the drying material (Afzal and Abe, 1998). The electric field increased the permeability of the cell membrane and as a result, the diffusion of water from the centre of the leave tissues to the drying surface was speeded up.

6.4. Effect of treatments on the quality of the final product

Moisture removal provokes microscopic and macroscopic structural modifications such as shrinkage and cell collapse that in turn reduces the mass transfer (Barat et al., 2001; Fito and Chiralt, 2003; Seguí et al., 2010). Shrinkage and tissue disintegration may also lead to texture and colour changes and lose of nutritional value; resulting in low quality dried products. Any application which modifies the cellular structure or speed up drying without considerable disintegration of the cellular tissue of the drying material is thus likely to influence the quality of the final product. Such effects were observed when trehalose was impregnated into the leaves prior to drying. The reduced porosity due to the incorporated solute offers some

protective action on the cellular structure of the product, which led to less tissue damage and better rehydration properties. Leave tissues that were reversible electroporated also had less tissue damage and had higher rehydration ratio compared to leave tissues that were irreversibly electroporated, though the later dried faster.

Consumers characterise dried products eaten after rehydration by their colour, texture, and shape resemblance to the raw material. Less colour changes and the ability of dried products to rehydrate are thus important quality indexes. The colour analysis shows that dried samples that were reversibly electroporated with the opened stomata electroporated were more bright, greener and resulted in less total colour change compared to all other dried samples. Wetting of dried products with water or with saliva during consumption should liberate nutritional parameters such as aroma which are important quality parameters. The loss of aroma in air-dried herbs is a major constraint to the marketing of dried herbs. These effects were observed in the untreated dried samples which exhibited a hay-like flavour perception but the samples that were reversibly electroporated with the opened stomata electroporated prior to drying exhibited a distinct oily flavour perception. Reversible electroporation of opened stomata thus offers the opportunity to conserve aroma compounds in dried herbs.

7. Concluding remarks

The opposite effects of ABA and FC on opening and closure of the stomata resulted in different drying times, confirming our assertion that stomatal aperture could influence drying time. Both irreversible membrane permeabilization at high field strength and the reversible membrane permeabilization of epidermal cells and opened stomata at low field strength improve mass transport in the leaf tissue and enhance the drying rate, thereby reducing the drying time compared to the control. Irreversible electroporation of the leaf tissue resulted in 47% reduction in the drying time, reversible electroporation of opened stomata resulted in 37% reduction in the drying time whilst leaves samples that were reversible electroporated but opened stomata was not electroporated resulted in 18% reduction in the drying time.

Vacuum impregnation with trehalose slightly improved the drying rate and rehydration properties but resulted in large difference in colour change whist the combination of vacuum infusion and PEF-treatment did not have any appreciable effect on the drying kinetics (resulted in large colour change but good rehydration properties). Optimization of PEF parameters to achieve stomatal electroporation is a difficult process but if attained, could enhance the drying efficiency even at low field strength.

The quality parameters analysed on the dried herb indicate that reversible electroporation of opened stomata resulted in less colour change, produced good rehydration properties and significantly improved the abundance of the aroma compounds relative to the untreated dried samples. Reversibly electroporation of opened stomata prior to drying thus represent the best treatment in this studies and indicates the potential of targeting stomata to enhance the rate of moisture removal during dehydration without irreversible damage of the leave cell membrane and conserve aroma in dried herbs.

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