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Grant Thamkaew

DOCTORAL DISSERTATION

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To be defended in Lecture Hall F, at the Center for Chemistry and Chemical Engineering (Kemicentrum) on Friday, 18 March 2022 at 09.00 a.m.

Faculty opponent
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Abstract

As commercial dried herbs are of lower quality than fresh herbs, it is of key importance to understand the effect of pre-drying treatments and drying techniques on the quality of the dried product. Several technologies are reviewed, focusing on their effects on aroma and color, with the goal of providing an overview of various technological strategies developed for improving the quality of aromatic herbs for industrial drying.

One of the pretreatments, pulsed electric field (PEF), can be used to enhance the drying rate of plant leaves. The electroporation of guard cells on the plant leaf surfaces results in sustained stomata opening during the drying process that, in consequence, increases the drying rate. The effect of electroporation parameters on the reversible permeabilization of cells in Thai basil leaves, specifically cells on the leaf surface, was investigated. Various PEF parameter combinations were used. Microscopic observations were used to assess the effect of these parameters on the electroporation of the leaf surface. The results showed that the electroporation of epidermal cells increased with increasing treatment time. After homogeneous electroporation of epidermal cells, guard cells were electroporated. Electroporation of epidermal cells on the leaf surface increased with voltage, pulse width, and number of pulses. Six specific PEF parameter combinations were found to electroporate the guard cells on the leaf surface while maintaining the leaves' viability.

In this study, one of the six established electroporation combinations (200 monopolar, rectangular pulses of 50 μs pulse duration, 760 μs between pulses, and nominal field strength of 650 V/cm) was used, followed by a 24-hour resting period in humid conditions before hot air drying at 40 °C. This treatment helped some cells in Thai basil leaves to survive different levels of dehydration (moisture ratio = 0.2 and 0.1). We show that resting after the application of reversible PEF may allow a hardening phase to exert a protective effect on the cells, thus reducing damage during subsequent drying. Cell vitality preservation would be associated with a more turgid and fresh-like rehydrated product.

Furthermore, the properties of dried and rehydrated Thai basil leaves were assessed with two different drying methods, convective drying at 40 °C and vacuum drying at room temperature. Vacuum drying caused more cell damage and tissue collapse than convective air-drying. Remarkably, reversible electroporation followed by resting resulted in greater trichome preservation, showing that this pretreatment protects trichomes even after complete dehydration (water activity, aw < 0.6).

Keywords stomata opening, trichome structure, stress response, viability preservation

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Doctoral Thesis

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MADE IN SWEDEN

Success isn’t defined by what you have received,
but what you’ve been able to maintain

Abstract

As commercial dried herbs are of lower quality than fresh herbs, it is of key importance to understand the effect of pre-drying treatments and drying techniques on the quality of the dried product. Several technologies are reviewed, focusing on their effects on aroma and color, with the goal of providing an overview of various technological strategies developed for improving the quality of aromatic herbs for industrial drying.

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Population scientific summary

Dried herbs' most important feature is their unique aroma. However, drying causes the essential oils in herbs to degrade, resulting in loss of aroma. The color of the herbs may also degrade due to heat during the drying process. Several novel drying techniques have been developed, and many of them have shown promising results for overcoming these issues. However, only a few have been implemented in the industry. This is because most developed drying techniques need a major overhaul of the drying system, which requires a significant investment.

Because heat is used for a long time during the drying process, it is a rather energy-intensive process. As a result, recent studies have focused on reducing drying time. Additionally, reducing the amount of time that herbs are exposed to heat may aid in the preservation of the aroma and color in dried herbs.

In this study, we reviewed the effects of various drying techniques on the color and aroma quality of dried herbs. We also looked at a few different pretreatments and how they affect the quality of dried herbs. Electrical treatment, one of the most effective pretreatments, was also investigated further in the thesis.

Electric treatment can be used to process foods in a variety of ways. The "Pulse Electric Field" or "PEF" process is used in this case. The method uses electric pulses to process herbs before drying. Electric pulses are generated and passed through herb leaves during the process, with the goal to create pores on the cell membranes; this process is called "electroporation". Electroporation can be either reversible (cells remain viable) or irreversible (cells die), depending on the intensity of PEF parameters. Irreversible electroporation is more effective at shortening drying times compared to reversible electroporation. However, the death of cells causes undesirable changes in the herbs, such as color changes and aroma loss. Reversible electroporation, on the other hand, is not as effective in reducing drying times but may result in better retention of color and aroma. This thesis shows that, in order to achieve a drying enhancement effect with reversible electroporation, the guard cells of the leaf's stomata should be electroporated.

Stomata are unique structures on the surface of plant leaves that control gas exchange between the leaf and the surrounding environment. Drought causes stomata to close, preventing the plant from losing moisture. As such, the stomata of plant leaves are closed during the drying process to prevent moisture evaporation, resulting in a slower drying rate. Stoma is made up of two guard cells, which are specialized cells and create a hole in between those two cells. The stomata open and close due to the actions of these cells. This thesis shows that when these cells are electroporated, they lose their ability to control the stomata aperture and they remain open during the drying process. As a result, the drying time is drastically reduced. When compared to untreated samples, samples with electroporated guard cells showed a drying time reduction of 70-80%.

However, since there are many parameters involved in the electroporation of cells such as pulse duration amount of pulses, and voltage of the electric pulse, our work started with the attempt to find the effect of each parameter on the electroporation of cells in Thai basil leaves. The findings of this study show that electroporation of the cells on the surface of the leaves increased with voltage, pulse duration, and pulse count. Our results also showed that guard cells were electroporated with higher intensity of PEF treatment than other cells on Thai basil leaves. With six specific parameter combinations established in the work, guard cells can be electroporated while the leaves remain viable.

We investigated the effect of reversible PEF treatments on the survivability of the cells in Thai basil leaf tissues and discovered that the treatments had the ability to increase cell survivability during the drying process if the treated leaves were rested in saturated moisture condition for 24 hours after electroporation. However, as the drying process progressed, this protective effect was limited to specific moisture levels. Furthermore, we have investigated the effect of the treatment when combined with another drying method, vacuum drying. However, vacuum drying caused more cell damage and tissue collapse than convective air-drying.

In terms of the aroma of the samples, we looked at the integrity of the oil glands on the surface of Thai basil leaves (called trichome) in completely dried samples and discovered that reversible electroporation followed by resting resulted in better trichome integrity of dried Thai basil leaves, indicating that this pretreatment still protects trichomes after complete dehydration.

Although, reversible PEF treatments are only a short additional processing step before the drying process, they have great potential to become a suitable quality and process efficiency improvement technique for the drying of herbs.

List of Publications

I. **Thamkaew, G**., Sjoholm, I., Gómez Galindo, F. (2021)
A review of drying methods for improving the quality of dried herbs
Critical Reviews in Food Science and Nutrition, 61(11), 1763-1786.

II. **Thamkaew, G**., Gómez Galindo, F. (2020)
Influence of pulsed and moderate electric field protocols on the reversible permeabilization and drying of Thai basil leaves
Innovative Food Science & Emerging Technologies, 64, 102430.

III. **Thamkaew, G**., Wadsö, L., Rasmusson, A. G., Gómez Galindo, F. (2021)
The effect of reversible permeabilization and post-electroporation resting on the survival of Thai basil (*O. Basilicum* cv. thyrsiflora) leaves during drying
Bioelectrochemistry, 142, 107912.

IV. **Thamkaew, G**., Rasmusson, A. G., Orlov, D., Gómez Galindo, F. (2022)
Reversible electroporation and post-electroporation resting of Thai basil leaves prior to convective and vacuum drying.
Submitted

The Author's Contributions to the Papers

I. The author gathered all relevant references, had critical discussions with the co-authors about their content and wrote the manuscript.

II. The author designed the experiments together with the co-authors. The author performed all the experiments, processed all the data, created all the figures, and wrote the paper along with contributions from the co-authors.

III. The author designed the study with suggestions from the co-authors, performed the experiments, evaluated the results and wrote the paper with minor contributions from the co-authors.

IV. The author designed the study and performed the experiments. The author evaluated the results in cooperation with the co-authors and wrote the paper.

Contents

Introduction and Objectives

Herbs are highly perishable foods due to their high moisture content. They are usually processed by drying to increase their shelf life (Orphanides, Goulas, & Gekas, 2015). Herbs are typically used in small amounts in food recipes, solely to impart their distinct flavor. Due to their shelf stability and flavor, herb drying is, probably, the most well-known type of herb processing. However, drying may cause the aroma and color to degrade, resulting in a lower quality of dried herbs when compared to fresh herbs (Diaz-Maroto, Perez-Coello, & Cabezudo, 2002). Convective drying, which uses heat from hot air to evaporate moisture in herbs, is the most common method of drying herbs. However, during the convective drying process, aroma compounds may be destroyed or altered. Furthermore, the drying process can take a long time (Jin, Mujumdar, Zhang, & Shi, 2017), resulting in long periods of heat exposure.

Many herb drying techniques have been developed, mostly to address the issue of the drying process's high energy consumption and to improve the quality of the dried products (Jin et al., 2017). However, most of the newly developed drying techniques require a significant modification of the manufacturer's current process set-ups. Therefore, pre-drying treatments have also been explored. Pretreatments can be used to speed up the drying process while preserving the quality of the dried herbs. Various pre-drying treatments, such as blanching (Di Cesare, Forni, Viscardi, & Nani, 2003), ultrasonic (Tiwari & Mason, 2012), and electroporation treatments (Kwao, Al-Hamimi, Damas, Rasmusson, & Gómez Galindo, 2016), have been developed.

Among all pretreatments of herb drying, electroporation has great potential to improve both the quality and the efficiency of the drying process due to its short processing time, low energy consumption and continuous process capability. Many types of dried products, such as potatoes (Liu, Grimi, Lebovka, & Vorobiev, 2018), carrots (Wiktor & Witrowa-Rajchert, 2019), and peppers (Won, Min, & Lee, 2014), have been reported to improve their drying rate and quality using electroporation. In this thesis, we investigated the effect of electroporation on the drying of Thai basil leaves.

The electroporation process can be reversible (cells remain viable) or irreversible (cell death). Irreversible electroporation can be used to effectively increase the drying rate of foods (Arevalo, Ngadi, Bazhal, & Raghavan, 2004). However, due to the death of cells in the herb tissues, irreversible electroporation may result in the loss of aroma and color of the dried herbs such as sweet basil (Kwao et al., 2016). Reversible electroporation, on the other hand, could be used to improve the drying rate if the guard cells of stomata on the leaf surface are electroporated. By electroporating guard cells, the stomata lose their function to control their aperture, resulting in sustained stomatal opening during the drying process, which facilitates moisture loss. The increased drying rate induced by the sustained stomatal opening and the maintaining of cell survivability of reversible electroporation would result in higher quality dried herbs than the irreversible electroporation (Kwao et al., 2016), provided that low drying temperatures are used.

Reversible PEF is expected to cause a temporary drastic loss of metabolic homeostasis due to transient membrane permeabilization, and cells may not have enough time to recover from the electric treatment if dehydration starts immediately afterwards. Therefore, a resting step after electroporation (24 hours under humid storage at room temperature) may allow electroporated samples to recover. The hypothesis tested in this thesis is that the recovery process may allow cells to develop protective mechanisms against the damaging effect of drying.

If damage and tissue collapse could be reduced and cell vitality could be at least partially restored upon rehydration, a more turgid and fresh-like rehydrated product would be achieved. Therefore, the ultimate goal of this thesis is:

To use reversible PEF as a pretreatment, reducing drying times and reducing tissue collapse and cell damage

The four papers presented in this thesis contribute with the knowledge needed to achieve the described ultimate goal with the following specific aims:

- To review the current status of herb drying pre-treatments and processes for improving the aroma and colour quality of dried herbs (**Paper I**),
- To identify a set of parameters that cause reversible electroporation of the leaves and electroporate stomatal guard cells (**Paper II**),
- To investigate the effect of post-electroporation resting on cell damage at certain levels of dehydration (**Paper III**).
- To compare the effect of reversible PEF and post-electroporation resting on fully dehydrated leaves dried with hot air and under vacuum (**Paper IV**).

Drying herbs – current state of the art

Paper I reviewed the effect of various pre-drying treatments and drying methods on the quality of dried herbs.

Pretreatments could be utilized to improve the drying rate and quality of dried herbs. Various drying pretreatments have been developed such as blanching, ultrasound, and pulsed electric field. The effect of pretreatments on different quality aspects of dried herbs is shown in **Table 1**.

Blanching is probably the most well-known drying pretreatment due to its ability to minimize color degradation of herbs (Di Cesare et al., 2003). Blanching could also be combined with a chemical treatment such as the addition of Ca^{2+} in the blanching water to improve the cell wall integrity of herbs such as Java leaves (Klungboonkrong, Phoungchandang, & Lamsal, 2018). Ultrasound, on the other hand, is well-known for its ability to increase the drying rate of various types of food material including herbs (de la Fuente-Blanco, Riera-Franco de Sarabia, Acosta-Aparicio, Blanco-Blanco, & Gallego-Juarez, 2006). Due to the faster drying rate, ultrasound-treated herbs such as parsley can be dried more quickly and result in better retention of color and bioactive compounds (Sledz, Wiktor, Nowacka, & Witrowa-Rajchert, 2017). Another pre-drying treatment is pulsed-electric field (PEF). The ability of PEF to aid the drying process by increasing the mass transfer of the treated food materials has been studied in many types of foods. The increased drying rate and very short processing time makes PEF a promising treatment to enhance the drying of heat sensitive foods. For drying herbs, PEF has shown to enhance the drying process of sweet basil leaves (Kwao et al., 2016). Reversible PEF showed better results to improve the color and aroma of basil leaves than the irreversible PEF. More details of reversible PEF treatments will be discussed further.

With regard to drying methods, due to its simplicity and controllability, convective drying is the most popular method used in the herb drying industry. The manufacturer can control process parameters such as temperature, time, air speed, and air humidity in order to achieve the desired product quality (Orphanides et al., 2015). However, many types of herbs have been found to suffer from significant color and chlorophyll degradation as a result of convective drying (Ahmed, Shivhare, & Singh, 2001; Di Cesare, Forni, Viscardi, & Nani, 2004; Negi & Roy, 2000). Furthermore, convective drying temperatures greater than 60 °C may result in significant losses of aroma and bioactive compounds in herbs (Deans, Svoboda, & Bartlett, 1991). As a result, for convective drying of herbs, low drying temperatures are recommended in order to preserve the major quality attributes such as aroma, color, and nutritional components (Shaw, Meda, Tabil, & Opoku, 2016). Microwave drying, freeze-drying, and fluidized bed drying are some of the drying methods that have been developed to overcome the problems of convective drying. **Table 2** summarizes the impact of these drying methods on the quality of dried herbs. Freeze-drying is another well-known drying method for heat-sensitive foods that is known to preserve color and aroma better than convective drying. Freeze-drying is a powerful herb drying process because it can dry herbs at very low temperatures (Antal, 2010). When compared to other drying processes, freeze dried herbs have better, if not the best, color and aroma quality in many types of herbs (Antal, 2010). In most of the aspects studied, including color, aroma, textural properties, and bioactive compound content, freeze-drying produced higher-quality dried herbs than convective drying (Orphanides et al., 2015). The low drying temperature and lack of oxygen during the drying process may be responsible for the minimal quality degradation caused by freeze-drying, which minimizes the oxidation of aroma compounds in dried herb essential oils and the degradation of chlorophyll (Pirbalouti, Mahdad, & Craker, 2013). However, the main disadvantages of freeze-drying are the high equipment investment costs and low process efficiency, as it is a batch process operation.

Figure 1 lists the methods reviewed in **Paper I** and highlights the methods investigated in this thesis

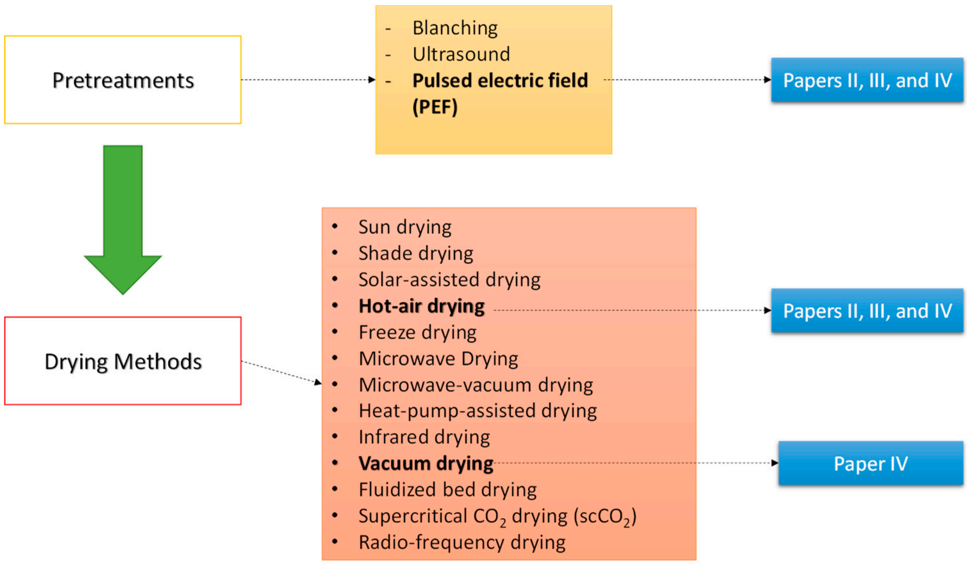

Figure 1. Schematic diagram of common pre-drying and drying methods for herbs and the techniques studied in this thesis

Table 1. Summary of the effects of pretreatments on the quality of dried herbs (Modified from **Table 2** in **Paper I**).

Pretreatment	Color	Chlorophyll content	Essential oil	Aroma compound profile	Structural properties	Bioactive content
Blanching	Improved color retention in many type of herbs (Singh, Raghavan, & Abraham, 1996)	Improved chlorophyll retention (Singh et al., 1996)	Data not available	Degradation of Aroma in some herbs such as Basil (Nani, Di Cesare, Viscardi, Brambilla, & Bertolo, 2001)	Improved cell wall integrity of dried Java leaves (Klungboonkrong et al., 2018)	Preserving bioactive compounds, such as lutein in parsley and sinensetin and eupatorin in Java tea. Decreased antioxidant content in some types of herbs (Klungboonkrong et al., 2018; Sledz, Wiktor, Rybak, Nowacka, & Witrowa-Rajchert, 2016)
Pulsed electric field (PEF)	Improved color retention (Kwao et al., 2016)	No data available	Enhanced the preservation of essential oil glands (Telfser & Gómez Galindo, 2019)	Improved aroma compound profile (only with reversible permeabilization) (Kwao et al., 2016)	Decreased cell collapsing when combined with air and vacuum drying. (Telfser & Gómez Galindo, 2019)	Data not available
Ultrasound	Data not available	Improved chlorophylls retention (Sledz et al., 2016)	Data not available	Data not available	Data not available	Preserving bioactive compounds, such as lutein in parsley (Dadan et al., 2018)

Table 2. Summary of the effects of drying methods on the quality of dried herbs (Modified from **Table 3** in **Paper I**).

Drying methods	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive content
Sun drying	Caused substantial color degradation (Arslan & Özcan, 2008, 2011)	No data available	Lower essential oil content compared to hot air and shade drying (Kumar, Sharma, Sharma, & Kumar, 2016)	Caused major degradation of aroma compound profile (Hanaa, Sallam, El-Leithy, & Aly, 2012)	Caused higher shrinkage compared to shade drying (Alara, Abdurahman, Abdul Mudalip, & Olalere, 2018)	Decreases antioxidant compound content (Arslan, Özcan, & Menges, 2010)
Shade drying	Better at preserving color of many types of herbs compared to sun drying, how air-drying, microwave drying, and freeze-drying (Demir, Gunhan, Yagcioglu, & Degirmencioglu, 2004)	Good retention of Chlorophyll content (Capecka, Mareczek, & Leja, 2005)	Better preservation compared to sun drying in many types of herbs (Hassanpouraghdam, Hassani, Vojodi, & Farsad-Akhtar, 2010)	Caused higher aroma profile alteration than hot-air drying (Rahimmalek & Goli, 2013)	Better at preserving trichome structure compared to hot-air and vacuum drying (Ebadi, Azizi, Sefidkon, & Ahmadi, 2015)	Showed good preservation of bioactive compound content in many types of herbs (Capecka et al., 2005)
Solar-assisted drying	No data available	No data available	Higher essential oil content compared to sun drying (Morad, El-Shazly, Wasfy, & El-Maghawry, 2017)	No data available	Better preservation of structures compared to hot-air drying (Klungboonkrong et al., 2018)	Better preservation of bioactive compound content compared to hot-air drying (Klungboonkrong et al., 2018)
Hot-air drying	Caused substantial color degradation (Diaz-Maroto et al., 2002)	Caused major chlorophyll degradation (Kathirvel, Naik, Gariepy, Orsat, & Raghavan, 2006)	Usually yields low essential oil content compared to other drying methods with some exceptions (Asekun, Grierson, & Afolayan, 2007)	Caused major degradation of aroma compounds profile especially with drying temperature higher than 60 °C (Pirbalouti et al., 2013)	Caused major degradation of dried herb structures especially with drying temperature higher than 60 °C (Alara, Abdurahman, & Olalere, 2019)	Caused major loss of bioactive compound content especially with drying temperature higher than 60 °C (Tummanichanont, Phoungchandang, & Srzednicki, 2017)
Freeze-drying	Excellent at preserving color of the dried products (Yousif, Durance, Scaman, & Girard, 2000)	Caused minor loss of chlorophyll content (Di Cesare et al., 2003)	Lesser loss of essential oil content compared to most drying methods (Ebadi et al., 2015)	Excellent at preserving aroma compound profile (Pirbalouti et al., 2013)	Structures of dried product are very well preserved (Klungboonkrong et al., 2018)	Excellent at preserving bioactive compound content (Klungboonkrong et al., 2018)
Microwave drying	Excellent at preserving color of dried products (Di Cesare et al., 2003)	Caused minor loss of chlorophyll content (Di Cesare et al., 2003)	Higher retention compared to hot-air drying (Pirbalouti et al., 2013)	Good preservation of aroma compound profile in most types of herbs (Calin-Sanchez, Lech, Szumny, Figiel, & Carbonell-Barrachina, 2012)	Better preservation of structures compared to hot-air drying (Therdthai & Zhou, 2009)	Better preservation of bioactive compound content compared to hot-air drying (Hamrouni-Sellami et al., 2012)

Microwave-vacuum drying	Excellent at preserving color of dried products (Yousif et al., 2000)	No data available	Caused higher loss of essential oil content than hot-air drying (Calín-Sánchez, Figiel, Lech, Szumny, & Carbonell-Barrachina, 2013)	Good preservation of aroma compound profile in most types of herbs (Calin-Sanchez et al., 2012)	Better preservation of structures compared to hot-air drying (Therdthai & Zhou, 2009)	Better preservation of bioactive compound content compared to hot-air drying and microwave drying (Therdthai & Zhou, 2009)
Heat-pump-assisted drying	No data available	No data available	No data available	No data available	Better preservation of structures compared to hot-air drying and solar-assisted drying (Klungboonkrong et al., 2018)	Better preservation of bioactive compound content compared to hot-air drying and solar-assisted drying (Klungboonkrong et al., 2018)
Infrared drying	Caused substantially higher color degradation compared to other drying methods (Naidu et al., 2016)	Caused more loss of chlorophyll content compared to other drying methods (Naidu et al., 2016)	Caused higher loss of essential oil content than hot-air drying with the exception of bay leaves (Naidu et al., 2016)	Showed good preservation of aroma compound profile in most type of herbs (Naidu et al., 2016)	No data available	Showed major loss in bioactive compound content (Torki-Harchegani, Ghanbarian, Maghsoodi, & Moheb, 2017)
Fluidized bed drying	Good color retention (Ceylan & Gurel, 2016)	No data available	No data available	No data available	No data available	Good preservation of bioactive compound content (Ceylan & Gurel, 2016)
Supercritical CO_2 drying ($scCO_2$)	Better at preserving color compared to hot-air drying (Michelino, Zambon, Vizzotto, Cozzi, & Spilimbergo, 2018)	No data available	No data available	No data available	Better preservation of structures compared to hot-air drying but less than freeze-drying (Michelino et al., 2018)	Better preservation of bioactive compound content compared to hot-air drying but less than freeze-drying (Michelino et al., 2018)
Radio-frequency drying	Caused major color degradation (Naidu et al., 2016)	Caused more degradation of chlorophyll content compared to hot-air drying (Naidu et al., 2016)	No data available	No data available	No data available	Caused more degradation of bioactive compound content compared to hot-air drying (Naidu et al., 2016)

Pulsed electric field as a pretreatment prior to drying

Pulsed electric field (PEF) is a non-thermal food processing technique that involves the electroporation of cell membranes in biological tissues, resulting in an increase in cell permeability (Barba et al., 2015). The effects of PEF have been studied in a wide range of foods, including meat (Bhat, Morton, Mason, Jayawardena, & Bekhit, 2019), fruits (Tylewicz et al., 2017), vegetables (Leong, Du, & Oey, 2018), and herbs (Kwao et al., 2016). Electroporation can be reversible (cells stay viable) or irreversible (cells die), depending on the severity of the protocol. In its irreversible form, PEF is most commonly used in food processing to inactivate microorganisms, increase extraction yield, and improve mass transfer.

Irreversible PEF has been used as a pre-drying treatment because it provokes the increase of cell permeability, which effectively increases mass transfer of biological tissues reducing drying time (Lebovka, Shynkaryk, & Vorobiev, 2007). When compared to other pre-drying treatments such as high-pressure processing and ultrasound treatment, PEF was reported to be the most effective in decreasing drying time and increasing water adsorption of air-dried apple (Wiktor, Landfeld, et al., 2021). Furthermore, PEF pretreatment was found to significantly reduce the energy consumption of carrot and apple drying processes (Wiktor, Parniakov, et al., 2021).

The permanent effect of increased permeability in irreversible electroporated tissues could be more effective in increasing the drying rate of food materials than reversible electroporation. However, (Kwao et al., 2016) reported that if the conditions are such that the stomatal guard cells are electroporated (**Figure 2** showing electroporation with propidium iodide staining) and remain open during the drying process, a reversible PEF pre-drying treatment could be used to reduce plant leaf drying time.

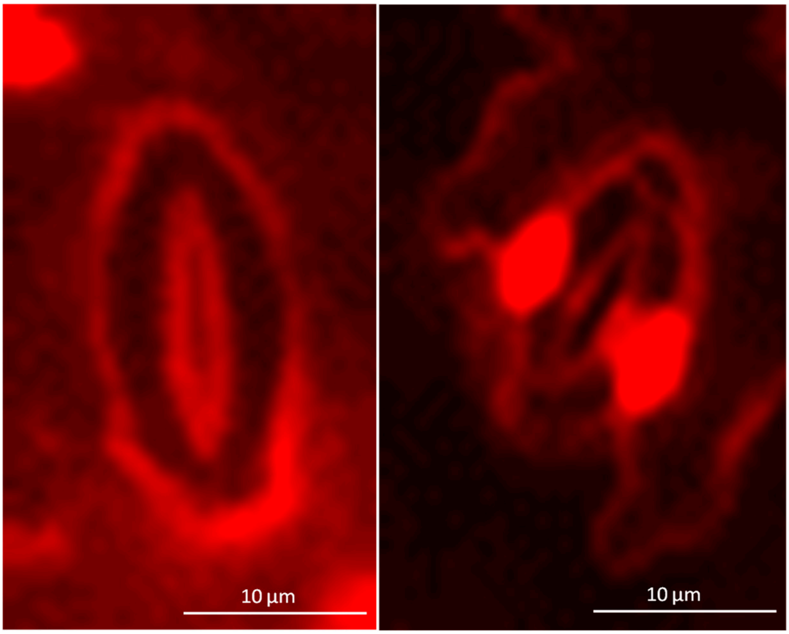

Figure 2. Representative micrographs of stomata found on Thai basil leaf surfaces. Left: non-electroporated guard cells, Right: electroporated guard cells. Electroporation is detected when propidium iodide penetrates the electroporated cells and stains their nucleous (corresponds to **Figure 3** in **Paper II**).

New findings

The level of each electroporation parameter, those that provoke reversible electroporation and guard cells electroporation in each type of herb, might be different due to their different biological properties, therefore, the effect of PEF parameters was investigated by systematically changing each PEF parameter: pulse width, pulse space, number of pulses, and voltage to find a combination of these parameters that could provoke the reversible electroporation of epidermal cells and electroporate the guard cells, specifically for Thai basil leaves **(Paper II)**.

Reversible electroporation and guard cell electroporation significantly reduced the drying time of Thai basil leaves (**Paper II,** in agreement with Kwao et al., 2016. However, Kwao et al. worked with drying at 50 ºC). The samples with electroporated guard cells had a drying time reduction of 70-80% when compared to untreated ones. Also, only epidermal cell electroporation induced by PEF (without electroporating the guard cells of stomata) can reduce the drying time by approximately 34% when compared to untreated samples (**Figure 3**).

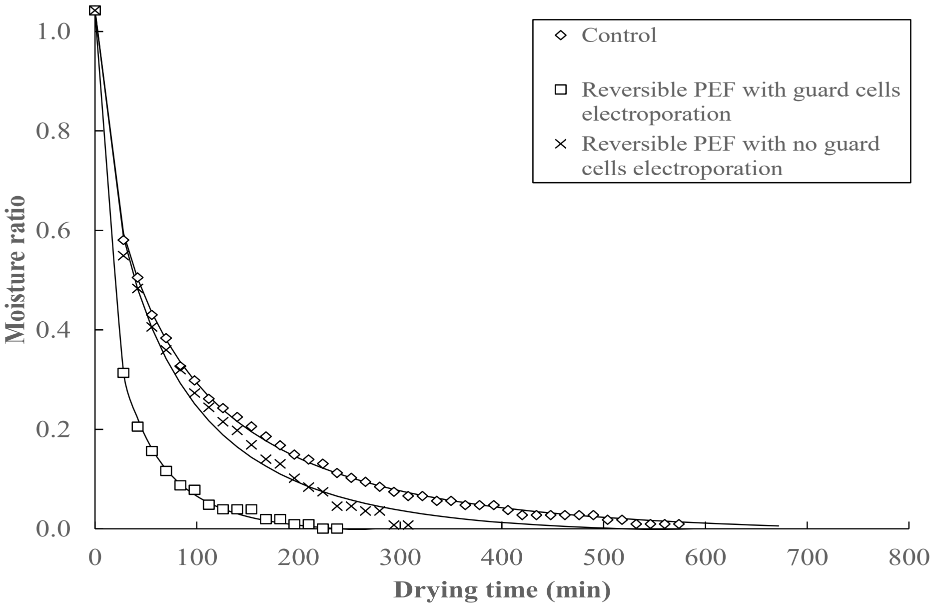

Figure 3. Drying curve of Thai basil leaf samples treated with reversible PEF and guard cells electroporation (square), without guard cells electroporation (cross), and control sample (rhombus). (modified from **Figure 12** in **Paper II**)

The results of **Paper II** show that the electroporation of cells on Thai basil leaf surfaces progressed with the increase of PEF parameter's intensity. As the electric field treatment intensity increases, the number of electroporated cells (as shown by propidium iodide staining of the cell nuclei) in the samples increases. **Figure 4** shows an example of this progression, where 650 V/cm and a pulse width of 50 μs were used with different numbers of pulses: 25, 100, 150, and 200. There were no electroporated nuclei on the leaf surface without the application of electrical treatment (**Figure 4A**). PEF protocol with 25 pulses caused electroporation of some epidermal cells on the leaf surface (**Figure 4B**), but no electroporation of guard cells was observed. The electroporated cells appeared at random on the leaf surfaces. When the number of pulses was increased to 100, the epidermal cells on the leaf surfaces permeabilized uniformly (**Figure 4C**), but no guard cells were found to be electroporated. The electroporation of some guard cells was observed when the number of pulses was increased to 150, (circles in **Figure 4D**). The guard cells on the leaf surfaces were homogeneously electroporated when the number of pulses was increased to 200 (circles in **Figure 4E**). The number of pulses required to achieve these levels of surface permeabilization was highly influenced by the voltage, pulse width, and pulse space applied to the samples.

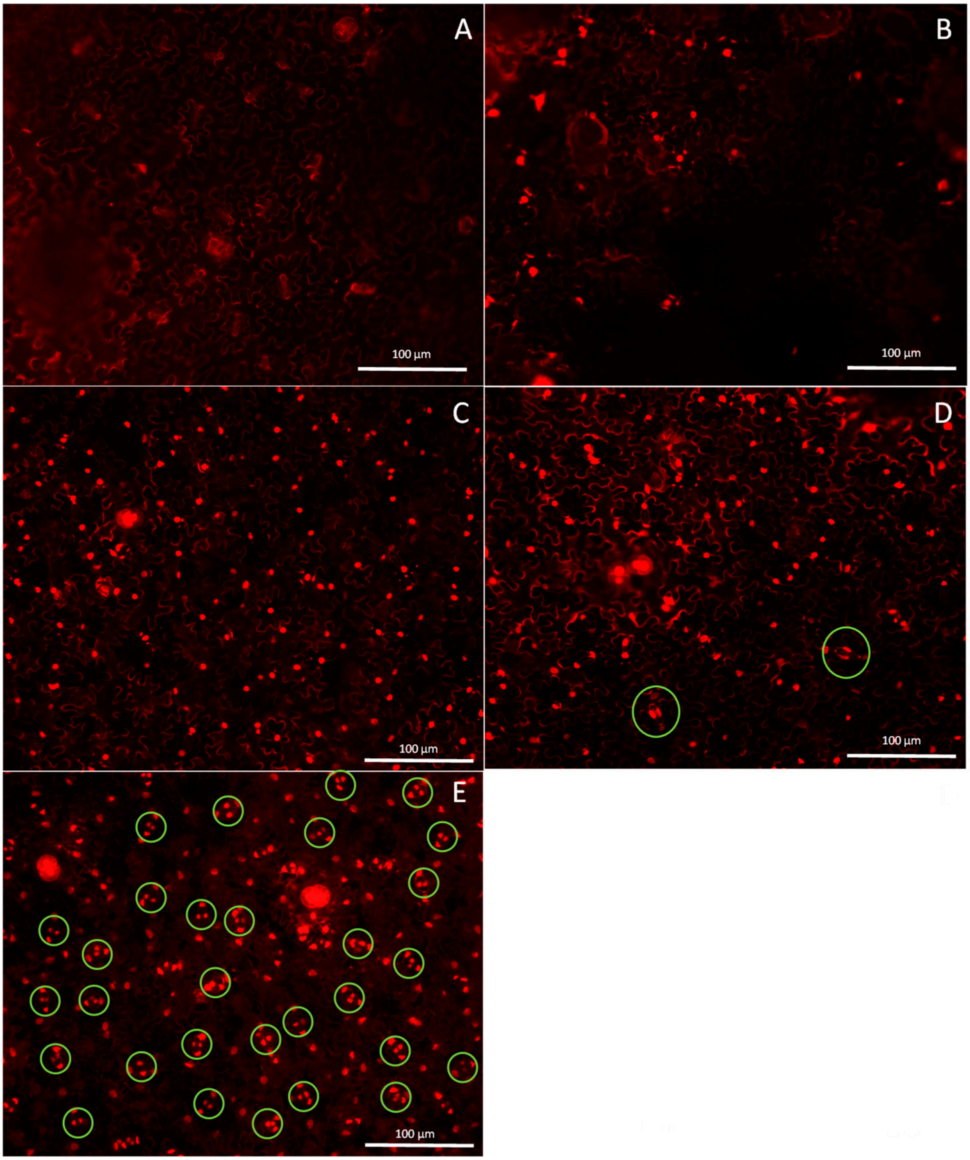

Figure 4. Representative micrographs of PEF-treated Thai basil leaf samples showing the permeabilization progression of cells on the leaf surface. Electroporation is shown by the staining of cell nuclei with propidium iodide. All samples were treated with monopolar pulses of 650 V/cm and a pulse width of 50 µs at a differing number of pulses. A: untreated sample, B: 25 pulses, C: 100 pulses, D: 150 pulses, and E: 200 pulses. Green circles in D and E indicate the electroporated guard cells (corresponds to **Figure 4** in **Paper II**).

By increasing the intensity of the parameters (increased in pulse width, number of pulses, voltage) and decreasing the space between pulses, more electroporation of epidermal cells and guard cells occurred. The effect of each PEF parameter tested in **Paper II** on the electroporation of epidermal cells and guard cells on Thai basil leaf surfaces is summarized in **Table 3**.

The "mildest" PEF protocol that could electroporate the guard cells homogeneously while maintaining the reversible electroporation (650 V/cm, 50 µs width, 760 µs space and 200 pulses) was chosen to be used for the investigations presented in **Paper III** and **IV**.

Table 3. Effects of PEF parameters (tested in **Paper II)** on the electroporation of epidermal and guard cells on Thai basil leaf surface.

Voltage (V)	Pulse width (µs)	Pulses space (µs)	Number of pulses	Homogeneous permeabilization of leaf surfaces achieved	Homogeneous permeabilization of guard cells achieved
100	250	760	500	x	
100	500	760	500	x	
100	150	760	1000	x	
300	250	760	200	x	
300	500	760	200	x	
300	150	760	500	x	
600	250	760	100	x	
600	150	760	200	x	
600	50	760	500	x	
650	250	760	25	x	
650	175	760	50	x	
650	150	760	75	x	
650	50	760	100	x	
650	175	380	100	x	x
650	175	1520	120	x	x
650	175	760	125	x	x
650	50	380	200	x	x
650	50	760	200	x	x
650	50	760	300	x	x

Metabolic consequences of reversible electroporation – effects on processing

The opening of pores in the plasma membrane caused by the application of PEF causes the efflux and influx of polar molecules. Then, the resealing process is accompanied by oxidative stress and the production of reactive oxygen species (ROS). As a consequence of membrane permeabilization, complex metabolic responses such as energy release from the movement of ionic species, adenosine triphosphate (ATP) hydrolysis to rebuild charge gradients across cell membranes, and other physiological events occurring during increased membrane permeability and long after resealing are active. The size and persistence of the pores created, i.e. the extent of membrane permeabilization, appear to be important determinants of metabolic responses (Gómez Galindo, 2017).

Upon reversible electroporation, cells recover and stress-induced metabolic responses may protect the cells against a further stress provoked by an industrial processing operation which, from a biological point of view, will mimic stress (Gómez Galindo, Sjöholm, Rasmusson, Widell, & Kaack, 2007). An example has been reported for freezing. Phoon et al. (2008) reported that the combination of vacuum impregnating trehalose, reversible PEF and resting (16 hours storage period between PEF and freezing) increased the freezing tolerance of spinach leaves. If the leaves were not rested before freezing, they did not develop freezing tolerance. The increased freezing tolerance of spinach leaves treated with both PEF and resting was also confirmed by Demir & Gómez Galindo (2018).

New findings

The effect of the application of reversible PEF and resting was tested for the drying of Thai basil leaves. Electroporated samples with and without resting were analyzed (**Paper III**) and two drying methods (convective and vacuum drying) were compared (**Paper IV**). **Figure 5** shows the rate of metabolic heat production (as measured with isothermal calorimetry) in PEF-treated samples during the resting period (24 hours) prior to drying compared with an untreated control. Throughout the resting period, the metabolic heat production (reported as specific thermal power) of the PEF-treated leaves is nearly three times that of the control. This elevated metabolism during resting indicates an increased mobilization of energy that may be an indication of stress responses.

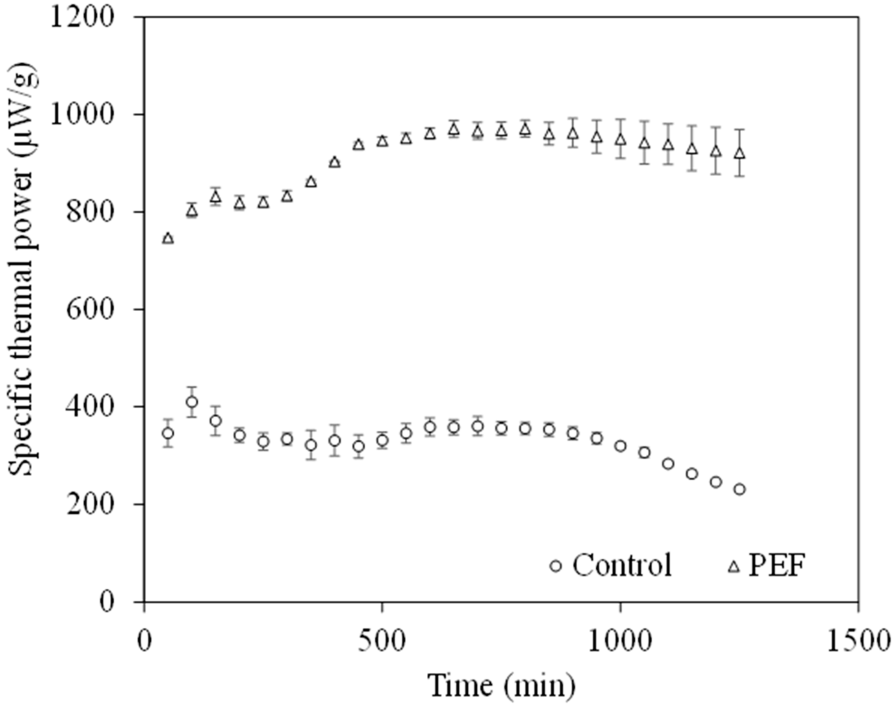

Figure 5 Heat evolution of Thai basil leaf samples treated with PEF (triangles) and control (circles) during the resting period (24 hours). Throughout the measurement, the leaf samples were given a constant supply of air. The average curves of three measurements are presented. The standard deviation of the mean is indicated by error bars in each data point.

When the leaves treated with reversible PEF and resting were dried in a convective oven at 40 ºC, certain levels of oxygen consumption (respiration) and photosynthesis at MR 0.2 and 0.1 were reported (**Figure 6**). The effect of PEF and resting on the survival of some cells in the tissue at MR = 0.1 (water activity = 0.61), while no cells survived in the control samples, is a remarkable result (**Paper III**).

The reversible PEF and resting treatment may allow some cells to elicit protective mechanisms that help them to withstand the drying process better than untreated leaves or leaves that were only PEF-ed but not rested before drying. In **Paper III**, apart from the respiration and photosynthesis data, evidence of cell viability was shown with different analysis: rehydration kinetics, ion release during rehydration, and vital staining of the rehydrated leaves.

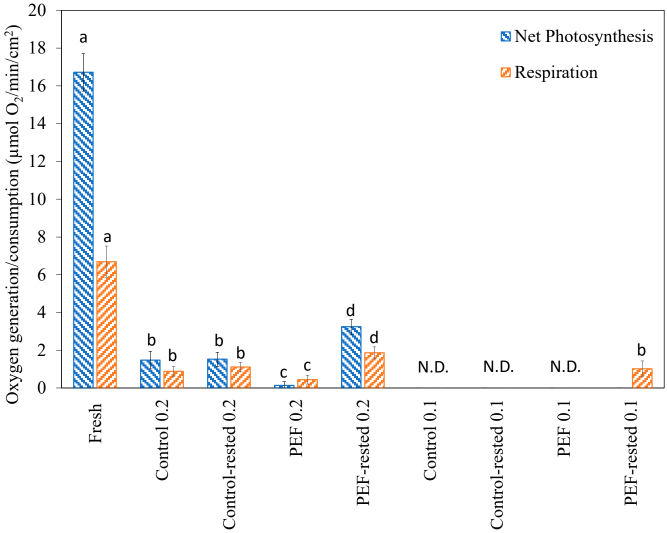

Figure 6. Oxygen generation of Thai basil leaf samples treated with control, control-rested, PEF, and PEF-rested and dried to the MR of 0.2 and 0.1 measured with light source on (photosynthesis), and oxygen consumption measured with light source off (respiration). N.D.: not detectable. Average values from 21 measurements are reported. Error bars in each data point represent the standard deviation of the mean. Different letter superscripts indicate statistically significant differences ($p < 0.05$) (corresponds to Figure 6 in **Paper III**).

Paper IV compared the effect of PEF and resting prior to convective and vacuum drying and evaluated the samples at different levels of moisture ratio, including the fully dehydrated leaves (MR = 0.05, water activity, aw = 0.5). At MR of 0.2 and 0.1, vacuum drying caused more cell damage than convective air drying, which can be seen in the higher ion release from the samples to the rehydrating water (**Figure 7**). Damage appears to be similar for both drying methods under complete dehydration (aw = 0.5). Resting after reversible PEF application had a protective effect only at high water activities (aw > 0.6).

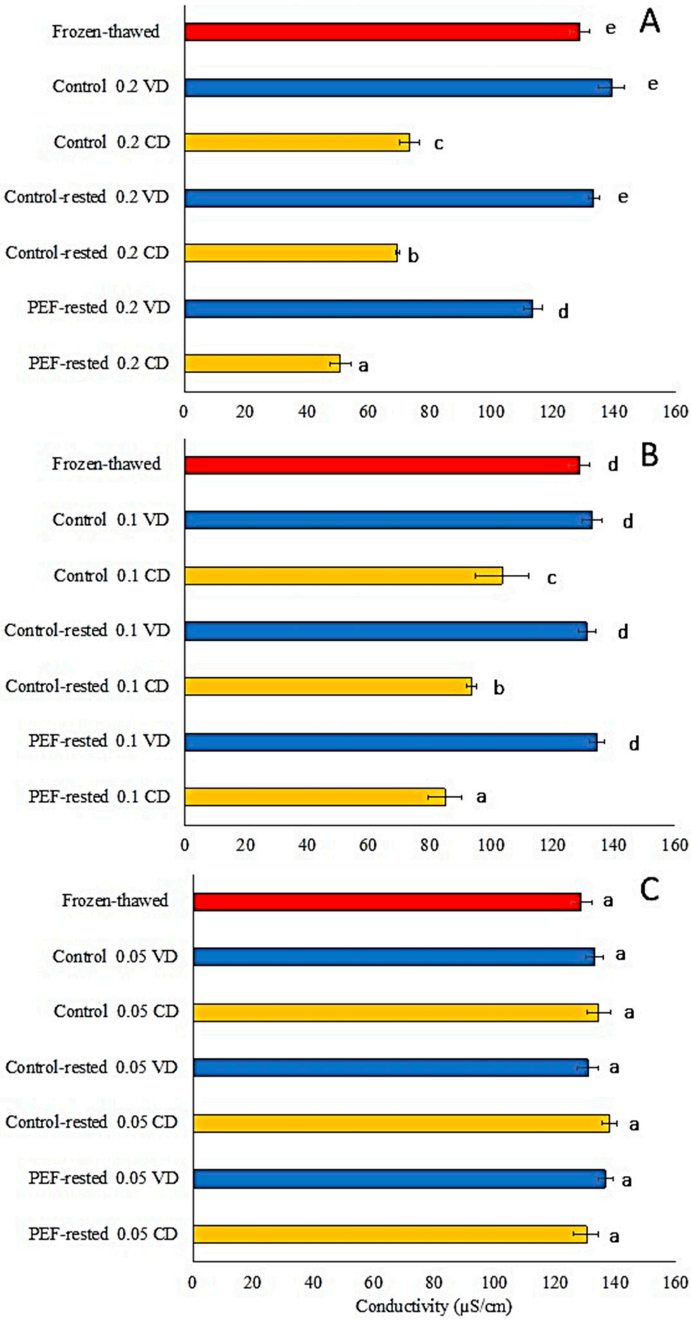

Figure 7. Electrical conductivity of rehydration water of dried Thai basil leaf samples subjected to different pretreatments (Control, Control-rested, and PEF-rested) and dried using two methods: convective drying (CD) and vacuum drying (VD) to the moisture ratio of 0.2 (A), 0.1 (B), and 0.05 (C). Average and standard deviation of 21 measurements are reported. Different letters next to the error bars indicate statistically significant differences ($p < 0.05$). (corresponds to **Figure 3** in **Paper IV**)

Regarding tissue integrity, **Paper IV** focuses on trichomes; leaf structures secreting and storing essential oils. A microscopy method was developed to investigate and measure the intact and deflated trichomes on the leaf surface using high accuracy optical microscopy. **Figure 8** shows trichomes with different levels of collapse found on Thai basil leaf samples. In fresh samples, most of the trichomes were fully inflated (**Figure 8A**), only small numbers of trichomes were found to be collapsed (**Figure 8B**). The intact trichomes in dried samples appeared slightly different than in fresh trichomes, noticeable partially deflated compared to intact fresh trichomes (**Figure 8C**). Also, partially inflated and collapsed trichomes were found in dried samples (**Figure 8D** and **8E**). The area of the trichomes were measured using the software of the microscope, as shown in **Figure 8F**.

When the area of trichomes was measured in the rehydrated samples dried by both studied drying methods, leaves treated with reversible electroporation followed by resting resulted in higher trichome preservation. The area of the trichomes was found to be similar to that of the fresh sample when the PEF-rested treatment was combined with convective drying (**Table 4**).

Table 4 Microscopic evaluation of trichomes on the investigated area of Thai basil leaves subjected to different pretreatments prior to drying with convective air drying (CD) at 40 ºC or vacuum drying (VC). The samples were dried to MR of 0.05 (corresponds to Table 4 in **Paper IV**).

Samples	Drying method	Partially collapsed trichomes (%)	Collapsed trichomes (%)	Area of trichomes (μm^2)
Fresh	CD	3 ± 2^{a}	0 ± 0^{a}	2267 ± 89^{a}
Control	CD	33 ± 5^{b}	18 ± 4^{cd}	1204 ± 133^{cd}
Control-rested	CD	27 ± 3^{c}	19 ± 5^{cd}	1001 ± 115^{cde}
PEF-rested	CD	20 ± 4^{d}	5 ± 3^{b}	2218 ± 65^{a}
Control	VD	32 ± 5^{b}	23 ± 3^{d}	727 ± 80^{e}
Control-rested	VD	29 ± 4^{c}	15 ± 3^{c}	827 ± 102^{de}
PEF-rested	VD	27 ± 5^{c}	7 ± 3^{b}	1785 ± 76^{b}

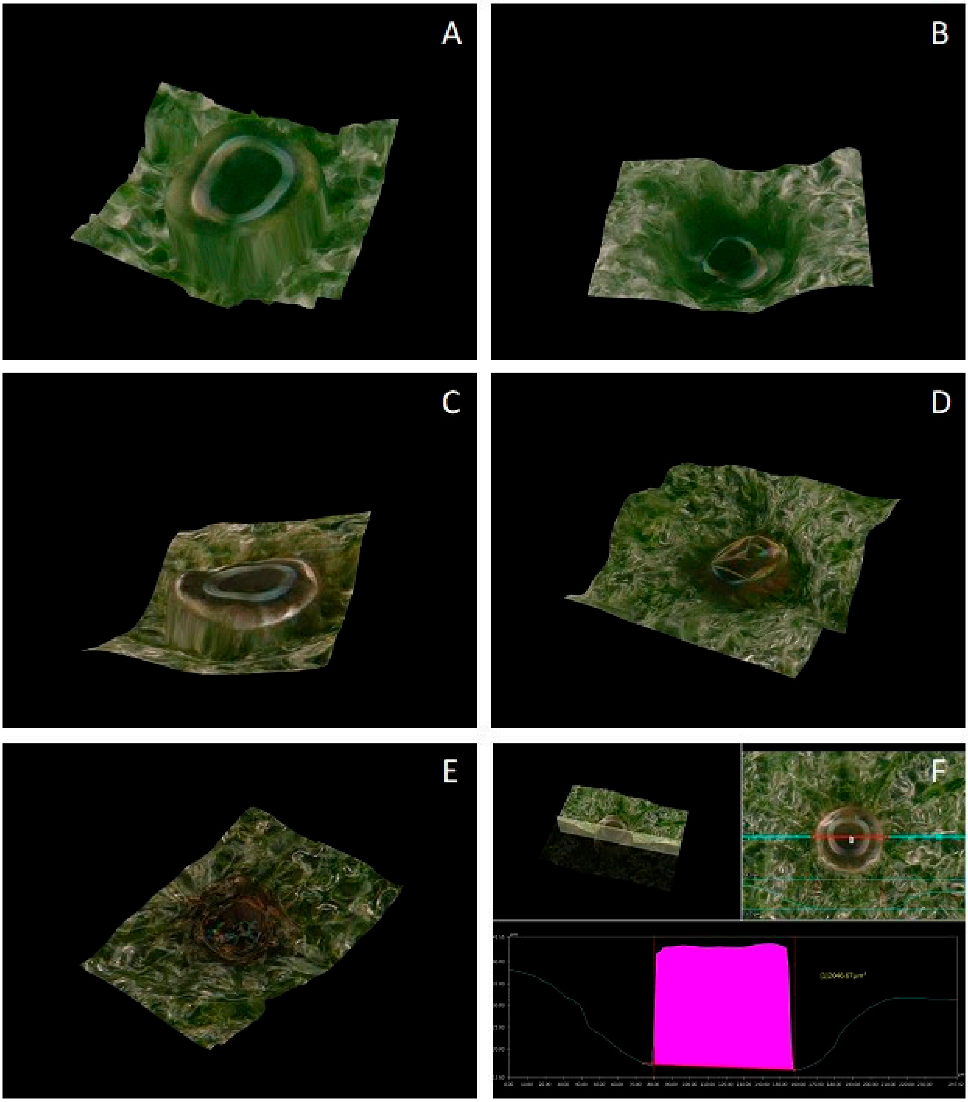

Figure 8 Fresh and rehydrated Thai basil leaves with different levels of intact and collapsed trichomes. Fully intact trichome in fresh samples (A), collapsed trichome in fresh samples (B), intact trichome in rehydrated samples (C), partially inflated trichome in rehydrated samples (D), collapsed trichomes in rehydrated samples (E), schematic of trichome area measurement using the microscope's built-in software, the measurement area shows the value of 2046.67 μm^2 (F). The image was acquired at a magnification of 700X, (corresponds to **Figure 7** in **Paper IV**)

Conclusions

The application of reversible PEF as pretreatment prior to drying of Thai basil leaves provokes opening of stomata and facilitates drying (**Paper II**). When the PEF treatment is followed by a 24-hour storage under humid conditions ("resting") some cells in the leaves remain viable after rehydration, showing decreased cell damage (**Paper III**). This effect was only evident in partially dehydrated leaves (Moisture Ratios 0.2 and 0.1; aw > 0.6), as cell damage in the fully dehydrated leaves was comparable with the untreated control and the samples that were frozen and thawed (**Paper IV**). However, reversible PEF followed by resting was shown to have a protective effect on trichomes upon complete dehydration, important anatomic structures for the quality of the leaves (**Paper IV**).

Other conclusions from each paper are presented below:

- The electroporation of cells on the surface of Thai basil leaves are progressive with the increase of the PEF parameter's intensity and guard cells required more intense parameters to be electroporated (**Paper II**).
- Electroporation of guard cells occurs within a narrow range of electroporation conditions, which are close to the limit between reversible and irreversible permeabilization (**Paper II**)
- Post-electroporation resting allows some cells in Thai basil leaves to recover and develop protective mechanisms for the upcoming drying process (**Paper III**).
- Vacuum drying caused more damage to cells in Thai basil leaves when compared to convective drying at the MR of 0.2 and 0.1. At MR = 0.05, the levels of damage for both drying methods were similar (**Paper IV**).

Future outlook

The research presented in this thesis adds to our understanding of the potential of reversible electroporation. However, future research in a number of important areas is required.

- More knowledge is needed on the protective mechanisms developed in cells hours after PEF treatment.
- The use of PEF and resting treatment in conjunction with other drying methods. It would also be necessary to explore the limitations of the treatment when combined with other drying methods.
- The effect of PEF-resting treatment on other types of herbs should be investigated.

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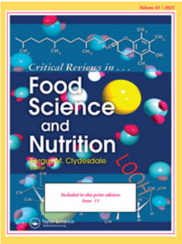
Paper I



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REVIEW

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A review of drying methods for improving the quality of dried herbs

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ABSTRACT
A large number of herb-drying studies have been conducted in recent decades and several herb-drying techniques have been introduced. However, the quality of commercial dried herbs is still lower than that of fresh herbs. In this paper, studies regarding the effect of drying techniques and pre-drying treatments on the aroma and color of dried herbs are reviewed with the aim of providing an overview of different technological strategies developed for improving the quality of aromatic herbs for their industrial drying.

KEYWORDS
Herbs; drying; pretreatment; aroma; essential oil; color

Introduction

Herbs are "any plant with leaves, seeds, or flowers used for flavoring, food, medicine, or perfume" (2019). Herbs are considered to be highly perishable foods due to their high moisture content and most herbs are chill-sensitive (Pirbalouti, Mahdad, and Craker 2013). They are therefore processed by drying to create shelf-stable products (Orphanides, Goulas, and Gekas 2016). Drying preserves the quality of herbs by reducing the moisture content, which inhibits the growth of microorganisms and chemical alterations during dried storage (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b). In the culinary sense, dried herbs are generally used as "flavoring" agents to add their characteristic aromas to the foods. Apart from the culinary usages of herbs, their essential oil can be used as an antimicrobial agent that is effective against bacteria, yeast, and molds (Bor et al. 2016). Dried herbs also have many applications in other fields, such as in medical and toiletry products and in perfume manufacturing. Herbs are known to be an excellent source of antioxidants (Embuscado 2015). The quality characteristics considered to be the most important for dried herbs may depend on their usage. For instance, the quality of medical dried herbs is defined by the content of bioactive compounds (Ebadi et al. 2015), while the quality of culinary dried herbs is usually defined by their color and fresh-like characteristic aroma (Rahimmalek and Goli 2013). The focus of dried-herb quality in this review will be on the color and aroma.

A large number of herb-drying studies have been conducted in recent decades and several herb-drying techniques have been introduced. Studies on herb-drying methods have received increased attention in the past 20 years. For example, when using the Web of Science with "herb" and the name of drying method as topics and "drying" as a title, an increasing trend of studies can be seen in different drying methods (Figure 1). Drying techniques have been developed that aim to improve quality as well as provide new possibilities to increase the efficiency of the drying process. Several drying techniques have been introduced in recent years, namely supercritical carbon dioxide drying (Busic et al. 2014) and heat-pump-assisted drying (Artnaseaw, Theerakulpisut, and Benjapiyaporn 2010). Besides the development of those drying techniques, the development of pre-drying treatments has also received considerable attention. A number of pretreatments for the drying of herbs have been studied during the past decades, such as ultrasound (de la Fuente-Blanco et al. 2006) and pulsed electric field (Kwao et al. 2016). Along with the developed drying methods and pretreatments, innovations have been introduced in solar-powered drying systems. Innovative integrated solar drying systems have been developed, such as heat-pump integrated solar dryers (Tham et al. 2017) and fluidized bed solar dryers (Ceylan and Gurel 2016). Hybrid drying, which combines two or more drying techniques have also been tested. Jin et al. (2018) reviewed several hybrid drying technologies such as solar-hot air drying, microwave-vacuum drying, and hot air- low humidity drying. Most of these developments aimed to decrease the drying time or lowering the drying temperature (Jin et al. 2018). The aim of this paper is to systematically review drying and pre-drying methods used for improving the quality of dried culinary herbs.

Quality characteristics of dried herbs

Dried culinary herbs are usually high in value, thus, the expectation of consumers regarding the quality of the product are generally high (Schaarschmidt 2016). The quality specifications of dried herbs have been listed mostly to ensure the chemical and microbiological safety of the products, such as, moisture content, bulk density, foreign matter, the content of excreta, aflatoxins and heavy metals. Table 1

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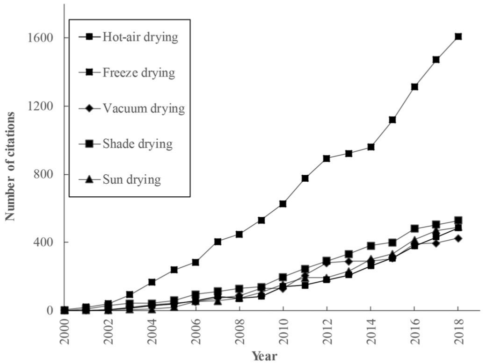

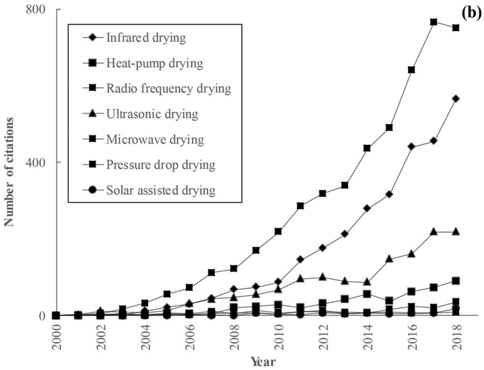

Figure 1. Number of citations by year of publication for drying herbs (2000–2019). (a) and (b) shows different drying methods. Source: Web of Science, using keywords "herb" and "[name of the drying method]" as topic. Accessed on 29 May 2019.

reports the drying technologies that have been used for various types of herbs as well as the quality properties that were analyzed. Among these quality properties, color and aroma are probably the most important quality characteristics affecting consumer acceptance (Schaarschmidt 2016). In this section, important aspects of aroma and color properties of the dried herbs will be reviewed.

Aroma compounds

Essential oil is the main contribution of herb aroma although it is present in small amounts (Rao et al. 1998). The International Organization for Standardization (ISO) has defined the meaning of the term "essential oil" as a "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase — if any — by physical processes" (ISO 9235:2013). Essential oils can be used in many types of applications, such as pharmaceutics, cosmetics, and the medical and food industries (Orphanides, Goulas, and Gekas 2016). In fresh herbs, essential oils are stored on the surface of the leaves in specialized structures called trichomes, which are uni- or multicellular appendages in the epidermal cells that develop outwards from the surface of plant organs such as leaves, roots or barks (Werker 2000). Upon drying, the retention of essential oils in the dried leaves depends on the integrity of the oil glands in the dried product (Ebadi et al. 2015). Therefore, preserving trichome integrity or minimizing the damage to trichomes during drying could improve the yield of essential oils and the aroma quality of dried herbs. Volatile compounds in herbs can be also found in glycosidically-bound forms as they are water soluble and can be accumulated in the plant tissues (Winterhalter and Skouroumounis 1997).

Chemical composition of essential oil and its alterations during drying

Essential oils are composed of a few or many chemical compounds, with some types of herbs containing more than a hundred chemical compounds (Antal et al. 2011). The chemical composition of the essential oils varies depending on the type of herb, harvesting season, postharvest practices, age of the plant and storage conditions (Dokhani et al. 2005). Each chemical compound contributes its specific flavor to the essential oil. This contribution relies on their specific odor threshold, which can be determined by the structure and volatility of the compound (Turek and Stintzing 2013). The changes in the concentration of the essential oil chemical components (either by chemical reactions or degradation), even with minor components, may result in drastic changes in the essential oil flavor (Grosch 2001).

Essential oil can be divided into 2 fractions: (1) the volatile fraction that yields about 90-95% of the total oil. This fraction is mainly composed of monoterpenes, sesquiterpenes, aldehydes, alcohols, and esters; and (2) the nonvolatile fraction, which contains hydrocarbons, sterols, and other large molecular weight molecules such as triterpenes, squalenes and saponins (Humphrey and Beale 2006; Orphanides, Goulas, and Gekas 2016). Some major chemical compounds of herbal essential oils have been reported, such as 1,8-cineole in bay leaves (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b), p-mentha-1,3,8-triene, β-phellandrene, and isopropenyl 4-methylbenzene in parsley (Diaz-Maroto, Perez-Coello, and Cabezudo 2002a); α-pinene, camphene, 1,8-cineole, camphor, bornyl acetate and borneol in rosemary (Rao et al. 1998); and α-pinene, β-pinene, 1,8-cineole, camphor, camphene, α-terpineol, caryophyllene, ascaridole and bornyl acetate in Iranian achillea species (Dokhani et al. 2005).

Many studies have been conducted to investigate the chemical profiles of essential oils. However, it should be noted that the methods for extraction and analysis methods of essential oils could influence the results (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b). For example, the extracted essential oil from dried bay leaves using simultaneous distillation extraction (SDE) contained α-thujene, camphene, β-pinene and elemicin, while the essential oil

Table 1. Analyzed properties for different herbs and drying methods.

Types of herbs	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive content	Source
Sun drying							
Acorus calamus L.			/	/			(Kumar et al. 2016)
Chamaemelum nobile L.				/			(Omidbaigi, Sefidkon, and Kazemi 2004)
Coriander sativum L.			/	/			(Pirbalouti, Salehi, and Craker 2017)
Cymbopogon citratus			/	/			(Hanaa et al. 2012)
Laurus nobilis L.	/						(Demir et al. 2004)
Mentha × piperita L.	/					/	(Arslan, Özcan, and Menges 2010)
Mentha longifolia L.			/	/			(Asekun, Grierson, and Afolayan 2007)
Ocimum basilicum L.			/	/			(Hassanpouraghdam et al. 2010)
Ocimum basilicum L.			/				(Tarakemeh, and Abutalebi 2012)
Ocimum basilicum L.			/	/			(Pirbalouti, Mahdad, and Craker 2013)
Ocimum basilicum L.							(Arslan, Özcan, and Unver 2005)
Rosmarinus officinalis L.	/						(Arslan, and Özcan 2008)
Satureja thymbra L.	/						(Arslan, and Özcan 2012)
Tanucetum parthenium			/	/			(Omidbaigi, Kabudani, and Tabibzadeh 2007)
Thymys daenensis subsp. daenensis.	/		/	/			(Rahimmalek, and Goli 2013)
Vernonia amygdalina					/		(Alara et al. 2018)
Shade Drying							
Acorus calamus L.			/	/			(Kumar et al. 2016)
Artemisia annua L.			/	/			(Khangholil, and Rezaeinodehi 2008)
Chamaemelum nobile L.				/			(Omidbaigi, Sefidkon, and Kazemi 2004)
Coriander sativum L.			/	/			(Pirbalouti, Salehi, and Craker 2017)
Cymbopogon citratus			/	/			(Hanaa et al. 2012)
Filipendula ulmaria L.						/	(Harbourne et al. 2009)
Laurus nobilis L.	/						(Demir et al. 2004)
Laurus nobilis L.				/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002b)
Laurus nobilis L.			/	/			(Sellami et al. 2011)
Lippia citriodora			/	/	/		(Ebadi et al. 2015)
Melissa officinalis L.						/	(Capecka, Mareczek, and Leja 2005)
Mentha × piperita L.						/	(Capecka, Mareczek, and Leja 2005)
Mentha × piperita L.	/	/	/				(Rubinskiene et al. 2015)
Mentha longifolia L.			/	/			(Asekun, Grierson, and Afolayan 2007)
Ocimum basilicum L.			/	/	/		(Díaz-Maroto et al. 2004)
Ocimum basilicum L.			/	/			(Pirbalouti, Mahdad, and Craker 2013)
Ocimum basilicum L.			/	/			(Hassanpouraghdam et al. 2010)
Ocimum basilicum L.			/				(Tarakemeh, and Abutalebi 2012)
Origanum vulgare						/	(Capecka, Mareczek, and Leja 2005)
Origanum vulgare	/	/					(Di Cesare et al. 2004)
Petroselinum crispum			/	/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002a)
Salix alba						/	(Harbourne et al. 2009)
Salvia officinalis L.						/	(Hamrouni-Sellami et al. 2013)
Tanucetum parthenium			/	/			(Omidbaigi, Kabudani, and Tabibzadeh 2007)
Thymys daenensis subsp. daenensis.	/		/	/			(Rahimmalek, and Goli 2013)
Vernonia amygdalina					/		(Alara et al. 2018)
Hot-air Drying							
Achilla frayrantissma L.			/	/			(Abaas, Hamzah, and Majeed 2013)
Acorus calamus L.			/	/			(Kumar et al. 2016)
Anethum graveolens L.	/						(Doymaz, Tugrul, and Pala 2006)
Anethum graveolens L.	/	/					(Kathirvel et al. 2006)
Anethum graveolens L.	/	/	/	/		/	(Naidu et al. 2016)
Anethum graveolens L.	/	/	/	/	/	/	(Naidu et al. 2016)
Anethum graveolens L.							(Pääkkönen, Malmsten, and Hyvonen 1989)
Anethum sowa Roxb.			/	/			(Raghavan et al. 1994)
Artemisia annua L.			/	/			

(*continued*)

Table 1. Continued.

Types of herbs	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive content	Source
							(Khangholil, and Rezaeinodehi 2008)
Artemisia dracunculus L.			/	/			(Arabhosseini et al. 2006)
Artemisia herb-alba.			/	/			(Abaas, Hamzah, and Majeed 2013)
Backhousia citriodora	/		/	/			(Buchaillot, Caffin, and Bhandari 2009)
Chamaemelum nobile L.				/			(Omidbaigi, Sefidkon, and Kazemi 2004)
Citrus hystrix D.C., Rutaceae			/	/			(Jirapakkul, Tinchan, and Chaiseri 2013)
Coriander sativum L.							(Ahmed, Shivhare, and Singh 2001)
Coriander sativum L.			/	/			(Pirbalouti, Salehi, and Craker 2017)
Coriander sativum L.	/	/					(Kathirvel et al. 2006)
Coriander sativum L.	/						(Shaw et al. 2016)
Cymbopogon citratus			/	/			(Hanaa et al. 2012)
Cymbopogon citratus	/						(Mujaffar, and John 2018)
Filipendula ulmaria L.						/	(Harbourne et al. 2009)
Foeniculum vulgare			/	/			(Gardeli et al. 2010)
Laurus nobilis L.	/						(Demir et al. 2004)
Laurus nobilis L.				/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002b)
Laurus nobilis L.							(Doymaz 2014)
Laurus nobilis L.			/	/			(Sellami et al. 2011)
Lippia berlandieri Schauer	/			/			(Yousif et al. 2000)
Lippia citriodora			/	/			(Shahhoseini et al. 2013)
Melissa officinalis L.			/	/	/		(Argyropoulos, and Muller 2014)
Melissa officinalis L.	/					/	(Rababah et al. 2015)
Mentha × piperita L.	/					/	(Arslan, Özcan, and Menges 2010)
Mentha × piperita L.							(Ashtiani, Salarikia, and Golzarian 2017)
Mentha × piperita L.			/	/			(Rohloff et al. 2005)
Mentha × piperita L.	/	/	/				(Rubinskiene et al. 2015)
Mentha × piperita L.							(Torki-Harchegani et al. 2017)
Mentha cordifolia Opiz ex Fresen	/				/		(Therdthai, and Zhou 2009)
Mentha longifolia L.			/	/			(Asekun, Grierson, and Afolayan 2007)
Mentha longifolia L.			/	/			(Asekun, Grierson, and Afolayan 2007)
Mentha spicata L.			/	/			(Antal et al. 2011)
Mentha spicata L.							(Doymaz 2006)
Mentha spicata L.	/	/					(Kathirvel et al. 2006)
Mentha spicata L.						/	(Orphanides, Goulas, and Gekas 2013)
Mentha spicata L.	/					/	(Rababah et al. 2015)
Ocimum basilicum L.				/			(Baritaux et al. 1992)
Ocimum basilicum L.				/			(Boggia et al. 2013)
Ocimum basilicum L.				/			(Calin-Sanchez et al. 2012)
Ocimum basilicum L.	/	/		/			(Di Cesare et al. 2003)
Ocimum basilicum L.			/	/	/		(Díaz-Maroto et al. 2004)
Ocimum basilicum L.			/	/	/		(Díaz-Maroto et al. 2004)
Ocimum basilicum L.			/	/			(Pirbalouti, Mahdad, and Craker 2013)
Ocimum basilicum L.			/	/			(Hassanpouraghdam et al. 2010)
Ocimum basilicum L.	/			/			(Kwao et al. 2016)
Ocimum basilicum L.							(Arslan, Özcan, and Unver 2005)
Ocimum basilicum L.	/	/					(Rocha, Lebert, and Martyaudouin 1993)
Ocimum basilicum L.			/				(Tarakemeh, and Abutalebi 2012)
Oreganum majorana L.			/	/			(Raghavan et al. 1997)
Origanum vulgare	/	/					(Di Cesare et al. 2004)
Petroselinum crispum Mill.			/	/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002a)
Petroselinum crispum Mill.	/						(Doymaz, Tugrul, and Pala 2006)
Petroselinum crispum Mill.	/	/					(Kathirvel et al. 2006)
Piper betle L.			/	/			(Pin et al. 2009)
Rosmarinus officinalis L.	/						(Arslan, and Özcan 2008)
Rosmarinus officinalis L.			/	/			(Piga et al. 2007)
Rosmarinus officinalis L.			/	/			(Rao et al. 1998)
Salix alba						/	(Harbourne et al. 2009)

(*continued*)

Table 1. Continued.

Types of herbs	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive content	Source
Salvia officinalis L.						/	(Hamrouni-Sellami et al. 2013)
Salvia officinalis L.	/					/	(Rababah et al. 2015)
Salvia officinalis L.				/			(Venskutonis 1997)
Satureja thymbra L.							(Arslan, and Özcan 2012)
Tanucetum parthenium			/	/			(Omidbaigi, Kabudani, and Tabibzadeh 2007)
Thymus officinalis L.			/	/			(Piga et al. 2007)
Thymus vulgaris L.			/	/			(Calín-Sánchez et al. 2013)
Thymus vulgaris L.	/					/	(Rababah et al. 2015)
Thymus vulgaris L.			/	/			(Sárosi et al. 2013)
Thymus vulgaris L.			/	/			(Venskutonis, Poll, and Larsen 1996)
Thymus vulgaris L.				/			(Venskutonis 1997)
Thymys daenensis subsp. daenensis.	/		/	/			(Rahimmalek, and Goli 2013)
Urtica dioica L.	/						(Alibas 2007)
Vernonia amygdalina					/		(Alara, Abdurahman, and Olalere 2019)
Freeze Drying							
Anethum graveolens L.							(Pääkkönen, Malmsten, and Hyvonen 1989)
Anethum sowa Roxb.			/	/			(Raghavan et al. 1994)
Coriander sativum L.			/	/			(Pirbalouti, Salehi, and Craker 2017)
Filipendula ulmaria L.						/	(Harbourne et al. 2009)
Foeniculum vulgare			/	/			(Gardeli et al. 2010)
Laurus nobilis L.				/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002b)
Lippia berlandieri Schauer	/			/			(Yousif et al. 2000)
Lippia citriodora			/	/	/		(Ebadi et al. 2015)
Mentha spicata L.			/	/			(Antal et al. 2011)
Mentha spicata L.						/	(Orphanides, Goulas, and Gekas 2013)
Ocimum basilicum L.	/	/		/			(Di Cesare et al. 2003)
Ocimum basilicum L.			/	/	/		(Díaz-Maroto et al. 2004)
Ocimum basilicum L.			/	/			(Pirbalouti, Mahdad, and Craker 2013)
Orthosiphon aristatus					/	/	(Klungboonkrong, Phoungchandang, and Lamsal 2018)
Petroselinum crispum			/	/			(Diaz-Maroto, Perez-Coello, and Cabezudo 2002a)
Salix alba						/	(Harbourne et al. 2009)
Salvia officinalis L.				/			(Venskutonis 1997)
Thymus vulgaris L.			/	/			(Calín-Sánchez et al. 2013)
Thymus vulgaris L.			/	/			(Sárosi et al. 2013)
Thymus vulgaris L.				/			(Venskutonis 1997)
Thymys daenensis subsp. daenensis.	/		/	/			(Rahimmalek, and Goli 2013)
Microwave Drying							
Anethum graveolens L.	/	/					(Kathirvel et al. 2006)
Coriander sativum L.			/	/			(Pirbalouti, Salehi, and Craker 2017)
Coriander sativum L.	/	/					(Kathirvel et al. 2006)
Coriander sativum L.	/						(Sarimeseli 2011)
Coriander sativum L.	/						(Shaw et al. 2016)
Laurus nobilis L.			/	/			(Sellami et al. 2011)
Levisticum officinale	/	/					(Sledz, and Witrowa-Rajchert 2012)
Mentha × piperita L.	/					/	(Arslan, Özcan, and Menges 2010)
Mentha × piperita L.	/	/	/				(Rubinskiene et al. 2015)
Mentha spicata L.	/	/					(Kathirvel et al. 2006)
Mentha spicata L.						/	(Orphanides, Goulas, and Gekas 2013)
Mentha spicata L.	/	/					(Sledz, and Witrowa-Rajchert 2012)
Ocimum basilicum L.				/			(Calin-Sanchez et al. 2012)
Ocimum basilicum L.	/	/		/			(Di Cesare et al. 2003)
Ocimum basilicum L.			/	/			(Pirbalouti, Mahdad, and Craker 2013)
Ocimum basilicum L.	/	/					(Sledz, and Witrowa-Rajchert 2012)
Oreganum majorana L.			/	/			(Raghavan et al. 1997)
Origanum vulgare	/	/					

(*continued*)

Table 1. Continued.

Types of herbs	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive content	Source
							(Sledz, and Witrowa-Rajchert 2012)
Petroselinum crispum Mill.		/					(Dadan et al. 2018)
Petroselinum crispum Mill.		/				/	(Dadan et al. 2018)
Petroselinum crispum Mill.	/						(Heindl, and Müller 2007)
Petroselinum crispum Mill.	/	/					(Kathirvel et al. 2006)
Petroselinum crispum Mill.	/	/					(Sledz, and Witrowa-Rajchert 2012)
Petroselinum crispum Mill.	/						(Sledz et al. 2016)
Petroselinum crispum Mill.	/						(Soysal 2004)
Rosmarinus officinalis L.	/						(Arslan, and Özcan 2008)
Rosmarinus officinalis L.			/	/			(Rao et al. 1998)
Salvia officinalis L.						/	(Hamrouni-Sellami et al. 2013)
Satureja thymbra L.							(Arslan, and Özcan 2012)
Thymys daenensis subsp. daenensis.	/		/	/			(Rahimmalek, and Goli 2013)
Urtica dioica L.	/						(Alibas 2007)
Microwave-Vacuum Drying							
Lippia berlandieri Schauer	/			/			(Yousif et al. 2000)
Mentha cordifolia Opiz ex Fresen	/				/		(Therdthai, and Zhou 2009)
Ocimum basilicum L.				/			(Calin-Sanchez et al. 2012)
Ocimum basilicum L.			/	/			(Yousif et al. 1999)
Petroselinum crispum	/						(Heindl, and Müller 2007)
Thymus vulgaris L.			/	/			(Calín-Sánchez et al. 2013)
Solar-assisted Drying							
Matricaria chamomilla L.							(Amer, Gottschalk, and Hossain 2018)
Mentha × piperita L.							(Morad et al. 2017)
Orthosiphon aristatus							(Gan et al. 2017)
Orthosiphon aristatus					/	/	(Klungboonkrong, Phoungchandang, and Lamsal 2018)
Heat Pump Drying							
Jew's mallow (unspecified specie)							(Fatouh et al. 2006)
Mentha spicata L.							(Fatouh et al. 2006)
Mint (unspecified specie)							(Aktaş et al. 2017)
Orthosiphon aristatus					/	/	(Klungboonkrong, Phoungchandang, and Lamsal 2018)
Pandanus amaryllifolius	/						(Rayaguru, and Routray 2010)
Petroselinum crispum							(Fatouh et al. 2006)
Infrared Drying							
Anethum graveolens L.	/	/	/	/		/	(Naidu et al. 2016)
Crocus sativus L.						/	(Torki-Harchegani et al. 2017)
Laurus nobilis L.			/	/			(Sellami et al. 2011)
Mentha × piperita L.							(Ashtiani, Salarikia, and Golzarian 2017)
Mentha × piperita L.	/	/	/				(Rubinskiene et al. 2015)
Salvia officinalis L.						/	(Hamrouni-Sellami et al. 2013)
Fluidized bed drying							
Ocimum basilicum L.	/		/	/			(de Aquino Brito Lima-Corrêa et al. 2017)
Mint (unspecified specie)							(Ceylan, and Gurel 2016)
Contact Drying							
Mentha × piperita L.	/		/				(Tarhan et al. 2011)
High power ultrasound-supercritical CO_2 Drying							
Coriander sativum L.	/						(Michelino et al. 2018)
Low-humidity hot-air Drying							
Anethum graveolens L.	/	/	/	/		/	(Naidu et al. 2016)
Radio Frequency Drying							
Anethum graveolens L.	/	/	/	/		/	(Naidu et al. 2016)
Rotary Drum Drying							
Mentha × piperita L.	/		/	/			(Tarhan et al. 2010)
Supercritical CO_2 Drying							
Ocimum basilicum L.	/	/	/	/		/	(Busic et al. 2014)
Vacuum Drying							
Anethum sowa Roxb.			/	/			(Raghavan et al. 1994)
Mentha × piperita L.	/	/	/				(Rubinskiene et al. 2015)
Urtica dioica L.	/						(Alibas 2007)

obtained from direct thermal desorption and solid-phase micro extraction (SPME) did not contain such compounds (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b). Some essential oil chemical components could be only artifacts of the extraction and analysis methods but not present in the fresh plants (Kubeczka 2009). Therefore, the extraction and analysis methods used need to be taken into consideration when comparing the amount or chemical compounds of essential oils. In addition, optimum sample preparation methods should be conducted to prevent the transformation of the analyzed components (Chen, Poon, and Lam 1998). Several essential oil extraction and analysis methods have been used, including hydro-distillation, solvent extraction or simultaneous distillation–extraction (SDE), and headspace methods (Lucchesi, Chemat, and Smadja 2004). Out of these methods, SDE and SPME are the most widely used (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b).

The chemical constituents of essential oils are unstable substances. They can be easily converted into other types of compounds though various chemical reactions such as oxidation, isomerization, cyclization, or dehydrogenation reactions. These chemical reactions can be triggered either enzymatically or chemically (Turek and Stintzing 2013). One of the most important chemical alterations of the essential oil constituents is autoxidation. The autoxidation reaction affects the deterioration process of terpenoids, which is the largest class of natural volatiles in plants (Başer and Demirci 2011). During the autoxidation of terpenoids, secondary products such as hydroperoxides can be formed and then decomposed in the presence of light, heat, and increased acidity in advanced stages of the oxidation process (Turek and Stintzing 2013). These chemical alterations of the essential oil constituents could occur during either the drying process or the storage period of the dried products. The utilization of heat during drying process could accelerate these chemical reactions (Lee, Lee, and Choe 2007). During the drying process, heat promotes the initial formation of free radicals, which catalyze the autoxidation process of the essential oil (Choe and Min 2006). Therefore, increasing drying temperature will lead to greater loss of aroma compounds and, consequently, more aroma quality degradation in the dried herbs.

The presence of light is another important aspect affecting the degradation of essential oils, especially during the sun-drying process, where herbs are exposed to direct sunlight, or during the storage of dried herbs without light protection packages. The presence of light, either ultraviolet or visible, accelerates the autoxidation process by triggering hydrogen abstraction, which leads to the formation of lipid alkyl radicals (Choe and Min 2006). Two types of oxygen molecules are responsible for the autoxidation of oil: the singlet oxygen ($^1O_2$) and the triplet oxygen ($^3O_2$). While $^1O_2$ is suggested to be mainly involved with the initial phase of the oil oxidation process (Lee and Min 1988), the $^3O_2$ is likely to react with the alkyl radicals at normal oxygen pressure and form lipid peroxyl radicals. These lipid peroxyl radicals are likely to abstract hydrogen from other molecules and catalyze the oxidation process, leading to the degradation of the aroma compounds. In addition, there are other aspects affecting the formation and the decomposition of hydroperoxides such as the presence of oxygen, antioxidants, water content, metal contaminants and chemical structure of the compounds (Turek and Stintzing 2013).

Drying can cause a great reduction in the amount of essential oil in many types of herbs, reportedly 36–45% in basil, 23–33% in marjoram, and 6-17% in oregano even when the herbs were air-dried at room temperature (Nykanen and Nykanen 1987), as cited in (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b). During the drying process, the volatile profile of the essential oil could change substantially due to the formation of secondary aroma compounds such as alcohols, aldehydes, peroxides, and ketones (Turek and Stintzing 2013). These secondary products may constitute a high percentage of the total volatile content in dried products. (Huopalahti, Kesalahti, and Linko 1985), reported that secondary aroma compounds might account for over 50% of the total volatile content in air-dried dill leaves (dried at 25, 40, and 50 °C). The changes in the essential oil compounds of herbs during the drying process might be a result of the release of compounds from the rupture of cell walls, oxidation reactions, or the hydrolysis of glycosylated volatile compounds (Xing et al. 2018).

The reduction or changes of the volatile compounds in dried herbs during the drying process depend on drying parameters including drying method, temperature, vacuum level (in case of processes such as vacuum drying or freeze drying), drying time, and amount of water evaporated during drying (Antal 2010; Figiel and Michalska 2016). In general, drying of herbs results in the reduction of volatile compounds and some drying methods might enable better preservation of the volatile compounds than others (Chua et al. 2019).

The drying temperature plays an important role on the preservation of volatile compounds of dried herbs after the drying process. Applying high drying temperature is commonly lead to the loss of volatile compounds content. At high drying temperature, trichomes may risk rupture which leads to the loss of volatile compounds through evaporation. In addition, high drying temperatures could promote the degradation of heat-labile compounds in the essential oil (Argyropoulos and Muller 2014). However, some contradictory results have been observed. In the case of hot-air dried lemon-myrtle leaves, drying temperature of 50 °C resulted in higher citral content compared to drying temperature of 30 and 40 °C. This better preservation effect might be caused by the crust layer which was formed on the leaves surface limiting the diffusion of high molecular weight volatile compounds from the tissues (Buchaillot, Caffin, and Bhandari 2009).

The vacuum level is one of the most important factors affecting the essential oil yield (Chua et al. 2019). In the case of freeze-dried spearmint, although decreasing the chamber pressure resulted in the decrease of drying time, it also caused a significant loss of volatile compounds (Antal et al. 2011). In the case of vacuum-microwave drying, increasing vacuum levels decreased the quality of volatile compounds of dried rosemary (Calín-Sánchez et al. 2011). The effects of these drying methods on the quality of dried herbs are reviewed in sections “Freeze drying” and “Microwave-vacuum drying” in this paper.

The amount of moisture evaporated from the tissues is another factor affecting the volatile compounds in dried herbs. In air-dried oregano, the amount of water evaporated was strongly correlated to the reduction of volatile compounds, as during the drying process water vapor might act as a carrier allowing the diffusion of volatile compounds from the tissues to the surroundings (Figiel et al. 2010). In addition, volatile compounds with high water affinity are more likely to be lost during the drying process (Sellami et al. 2011).

The changes in volatile compounds during the drying process also depend on the biological factors of the herbs, including initial moisture content, the age of the plant, growth conditions and harvesting time (Ascrizzi, Fraternale, and Flamini 2018). Storage conditions also affect the content of volatiles of the dried products, especially in the presence of light and oxygen (Baritaux et al. 1992). The reduction of some essential oil components can be considered to be a benefit, such as the reduction of pulegone, a hepatotoxin in Hedeoma pulegioides and Mentha pulegium (Asekun, Grierson, and Afolayan 2007; Chen, Lebetkin, and Burka 2001) reported that pulegone content in dried wild mint (Mentha longifolia L. subsp. capensis) was significantly reduced by hot-air drying at 40 °C. It has therefore been suggested that this type of mint be consumed in dried form rather than as fresh.

Color of dried herbs

The main objective of many herb-drying studies has been to improve the color of the dried products or reduce the color changes during drying and during storage (Baritaux et al. 1992). The color degradation in dried herbs is provoked by the degradation of pigments such as chlorophyll and anthocyanin. For green herbs, chlorophylls degradation is the most common change that may occur during the drying process (Rayaguru and Routray 2010). Lafeuille et al. (2014) analyzed chlorophylls and its colored derivatives in culinary herbs influenced by various drying processes. In the paper, a chlorophyll degradation ladder was designed to assess the dried herbs color after the drying process. The ladder was separated into four categories by the amount of green pigments preserved after the drying process: (1) no significant impact ($>$ 90% preserved), (2) low impact (65–90% preserved). (3) medium impact (35–65% preserved), and (4) important impact ($<$ 35% preserved). According to these criteria, freeze drying can be categorized into the first ladder as it showed no significant impact on the content of green chlorophyll derivatives. The most popular drying method, hot-air drying, was categorized into the second ladder. Sun drying falls into the forth category due to its significant impact on the preservation of green chlorophyll derivatives. Heat degradation pathways of chlorophyll have been described (Di Cesare et al. 2003).Two major types of chlorophylls are responsible for the changes in herb color during the drying process: chlorophylls *a* and *b*. The chemical structures of the two chlorophylls are very similar, with the only difference being that chlorophyll *b* has an aldehyde group at the C7 position of its porphyrin ring. The color of chlorophyll *a* is blue green, while chlorophyll *b* is yellow-green. Due to the asymmetry at carbon C13, chlorophylls *a* and *b* might turn into their epimers chlorophyll a' and b' under mild process conditions. These epimers have almost exact visible spectrum to their non-prime forms and does not affect the color of the dried products. However, the prime (′) epimers are slightly less stable than their original form (Scheer 1991), therefore, chemical reactions might occur easier than non-prime epimers. The changes or removal of chlorophyll molecule periphery might create derivatives with the same chlorophyll visible spectrum. The most common changes in this group is the loss of phytol group at C17 due to hydrolytic reaction catalyzed by enzymes in plants such as chlorophyllase (Lafeuille, Lefevre, and Lebuhotel 2014). Chlorophyllide which is a derivative from the loss of phytol from the chlorophyll molecule has the same visible spectrum as chlorophyll, however, it has higher water solubility and could be lost easily during heating processes such as blanching, which is one of the most common pre-drying treatment. Figure 2 shows the degradation pathways of chlorophyll during the drying process. The loss of chelated Mg^{2+} from chlorophyll structure creates olive-brown pheophytin. Process steps which damage the cell membrane, such as harvesting, heating, or drying could allow sap acidic compounds to react with chlorophyll molecules and promotes the loss of chelated Mg^{2+}. Chelated Mg^{2+} could be lost by both dry and moist heat and also occurred with external acid conditions (Scheer 1991). The loss of chelated Mg^{2+} is one of the most common color degradations of herb during drying process. In addition, chlorophyllide is more heat sensitive than chlorophyll in terms of losing its Mg^{2+}, the loss of chelated Mg^{2+} from chlorophyllide molecules creates olive-brown pheophorbide. The loss of phytol group from the chlorophyll *a* structure by heat occurs easier than the loss of Mg^{2+} (Di Cesare et al. 2003; Eskin 1990). As chlorophyll *a* is more sensitive to heat than chlorophyll *b*, the degradation of chlorophyll *a* result in a change in the chlorophyll *a*/*b* ratio, which changes the color of the dried products from green-blue to green-yellow.

Collapsing during the drying process of the plant tissues could lead to the release of chlorophyll molecules from the protein complex, which could promote the transformation of chlorophylls into pheophytins due to greater exposure of the chlorophylls' structure to heat. This event could also lead to the releasing of substrates for enzymatic browning reactions to the surrounding areas.

The degradation of chlorophylls *a* and *b* also depends on the type of plant. It has been shown that dried lovage and parsley, which are herbs of the *Apiaceae* family, showed higher retention of chlorophylls *a* and *b* in comparison with basil, mint and oregano, which are herbs of the Lamiaceae family (Sledz and Witrowa-Rajchert 2012). The changes in color could be reduced by optimizing the drying process parameters such as drying temperature, time, and air velocity. Pretreatments prior to drying, such as blanching (Di

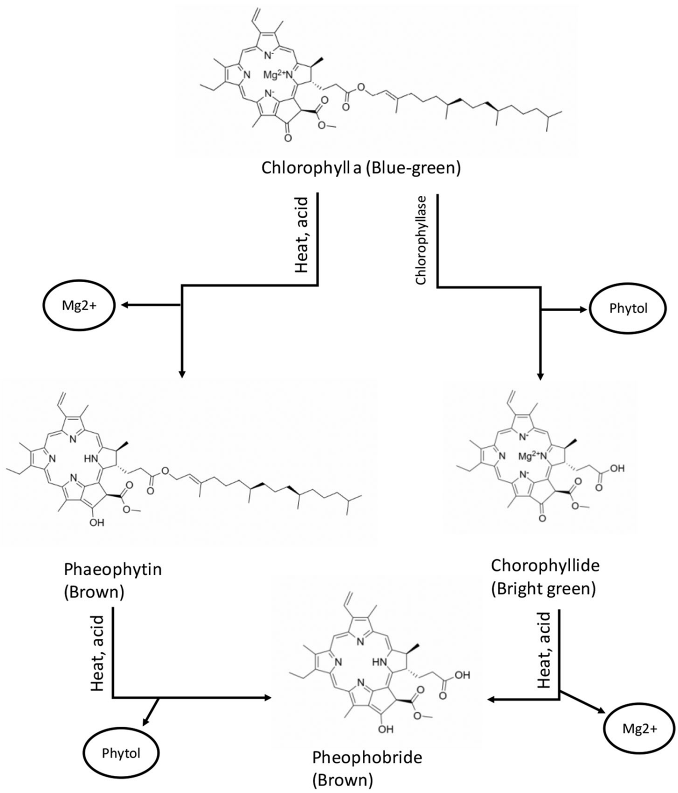

Figure 2. Chlorophyll degradation pathways during the drying process as described in Di Cesare et al. (2003). Chemical structures were taken from PubChems's database (Kim et al. 2019) and the structures were recreated using ChemDraw Cloud (version. 18.1.0-14 + eea6052, PerkinElmer, Waltham, MA, USA).

Cesare et al. 2003) and pulsed electric field (Kwao et al. 2016), were reported to improve the color of the dried herbs.

Pretreatments for drying of herbs

Pretreatments prior to drying are processing strategies aimed at achieving high-quality dried herbs, shorten the drying time, and reducing the energy consumption (Deng et al. 2019). Good pretreatments implementation should create only minimal modification to the drying process settings to reduce the follow-up costs from the modification (Rooy 2012). Table 2 summarizes studies reviewed in this section on the effect of pretreatments on the quality of dried herbs. Several pretreatments have been reported to provide benefits for drying of herbs, such as blanching, pulsed electric field, and ultrasonic treatment. In this section, the effect of pretreatments on the quality of the dried herbs prior to various drying methods will be reviewed.

Blanching

Blanching provides benefits to the drying of many types of herbs. The major benefit of blanching is the reduction of color degradation. It was reported that blanching reduced the drying time of basil. Steam blanching for 15 s increased the drying rate by a factor of 10 compared to untreated leaves (Rocha, Lebert, and Martyaudouin 1993). The steam-blanched dried basil leaves also showed better color retention and increased chlorophyll *a*/*b* ratio. Similar results were observed in parsley leaves; the steam-blanched parsley showed a 30% faster drying rate and energy consumption was reduced by 72% in comparison with the drying of untreated leaves. Also, the blanched dried parsley showed good lutein content retention and better color retention (Sledz et al. 2016). A similar result was obtained in dill leaves blanched in hot water for 1 min prior to drying with several drying methods, including through-flow drying (45 °C), cross-flow drying (40 °C), vacuum drying (45 °C), and freeze drying (Raghavan et al. 1994). Blanching decreased the drying time for all drying methods tested.

Table 2. Effects of pretreatments on the quality of dried herbs.

Pretreatment	Color	Chlorophyll content	Essential oil	Aroma compound profile	Structural properties	Bioactive content
Blanching	Improved color retention of dried dill in combination with hot-air drying, vacuum drying, and freeze drying. Improved also in basil, coriander, and parsley dried with hot air.	Improved chlorophyll content in many dried products such as basil and parsley in combination with hot-air drying.	Decreased essential oil content in dill in combination with hot-air drying, vacuum drying, and freeze drying.	Degradation of aroma in basil when combined with hot-air, microwave, and freeze drying.	Improve cell wall integrity of dried Java leaves dried with heat pump dehumidify drying but increase the drying damage to the structure of trichomes in basil dried with hot air.	Preserves bioactive compounds such as lutein in parsley dried with how-air and sinensetin and eupatorin in Java tea dried with convective drying, heat pump dehumidified drying, mixed-mode solar drying, and freeze drying.
Pulsed electric field (PEF)	Improved color retention of dried basil prior hot-air drying.	No data available	Enhanced the preservation of trichomes in basil prior hot-air and vacuum drying.	Increased retention of aroma compounds of basil dried with hot air (only with reversible permeabilization)	Decreased cell collapsing of basil when combined with hot air and vacuum drying.	No data available
Ultrasound	No data available	Improved chlorophyll retention in parsley dried with hot air	No data available	No data available	No data available	Preserved bioactive compounds, such as lutein in parsley dried with hot air

However, it was reported that blanching caused higher loss of the total essential oil content in the dried products. The opposite results were observed in blanched coriander leaves (80 °C in water), where the drying rate was slower in comparison with untreated leaves (Ahmed, Shivhare, and Singh 2001). Nevertheless, blanching resulted in better chlorophyll retention and higher rehydration capacity of the dried products compared to un-blanched dried products. Similar results were observed in basil (Nani et al. 2001). Using blanching in combination with chemical agents could provide benefits to the dried products; adding potassium metabisulfite to the blanching solution improved the retention of ascorbic acid, beta-carotene, and chlorophyll of dried amaranth and fenugreek leaves (Negi and Roy 2000).

There are different blanching techniques for herbs, such as water blanching, steam blanching, and microwave blanching (Singh, Raghavan, and Abraham 1996). The blanched dried marjoram and rosemary treated with all these blanching techniques showed better color retention in comparison with un-blanched dried products. In addition, water blanching showed the best color retention, followed by microwave and steam blanching. However, microwave blanching showed higher ascorbic acid content and better textural properties. Vacuum blanching, where the herbs are packed in a vacuum bag and then blanched in hot water, provided better retention of bioactive compounds in the dried product. The effect of water blanching and vacuum blanching (at 100 °C) on the quality of dried java tea prior to convective drying, heat pump dehumidify drying, mixed-mode solar drying, and freeze drying has been reported (Klungboonkrong, Phoungchandang, and Lamsal 2018). The results showed that vacuum blanching resulted in higher contents of sinensetin and eupatorin in the dried product in comparison with water blanching. Also, the vacuum-blanched dried leaves (dried using heat pump dehumidify dryer) showed better cell wall integrity than un-blanched samples.

Blanching can also be detrimental to herb quality. It has been reported to cause significant loss of the antioxidant properties in some types of herbs such as clove basil, *Basella alba*, *Corchorus olitorius*, and *Solanum macrocarpon* (Oboh 2005). Moreover, blanching was also reported to cause the degradation of aroma in some types of herbs such as basil, where the destruction of oil glands was observed. Blanching caused higher loss of aroma compounds in samples dried with several drying methods including air drying (50 °C), microwave drying, and freeze drying. The un-blanched-freeze dried sample was the only sample that showed no reduction in aroma compounds.

Pulsed electric field (PEF)

Pulsed electric field (PEF) is a non-thermal processing method that applies an external electric field to cells or tissues, provoking poration of the cell membrane. PEF has gained extensive attention due to its wide application range in food processing, such as extraction, drying, and microbial inactivation (Khan et al. 2018). Several studies on the effect of PEF on the drying of several types of plant raw materials have been conducted (Huang et al. 2018; Kwao et al. 2016; Ostermeier et al. 2018; Parniakov et al. 2016; Telfser and Galindo 2019). Most of these studies focused on the use of irreversible permeabilization (cells do not survive the application of PEF) of plant tissues, provoking permanent damage to the cell membrane and resulting in increased moisture diffusion coefficient and drastic reduction in drying time. This reduction in drying time could provide favorable results in the drying of heat-sensitive foods such as herbs (Orphanides, Goulas, and Gekas 2016). To the best of our knowledge, only two studies (Kwao et al. 2016; Telfser and Galindo 2019) have investigated the effect of reversible permeabilization (cells survive the application of PEF) as a pretreatment for drying on the quality of herbs. Both reversible and irreversible electroporation were able to electroporate guard cells of the stomata of basil leaves (Kwao et al. 2016). The electroporation of guard cells provoked sustained stomatal opening during the hot-air drying process, which increased the drying rate and improved color, aroma, and rehydration capacity of treated samples. Telfser and Galindo (2019) studied the effect of reversible permeabilization in combination with different drying processes (hot-air drying, vacuum drying, and freeze drying) on the quality of basil. This study showed that the reversible permeabilization treatment of the tissues reduced the drying time for every tested drying method (57% for hot-air drying, 33% for vacuum drying, and 25% for freeze drying). Moreover, reversibly PEF-treated leaves showed better preservation of trichome integrity with both hot-air and vacuum drying in comparison with untreated leaves. However, the trichomes of freeze-dried samples were damaged in both PEF-treated and untreated leaves.

Ultrasound

Ultrasound is a non-thermal pretreatment for drying of food materials. The process is conducted by applying high-power ultrasound with low frequencies (20–100 kHz) and high intensities (10–1000 W/cm) to the food material, resulting in increased mass transfer without heating or with only very subtle heating (Tiwari and Mason 2012). Ultrasound induces the formation of micropores on the surface of the materials, which results in a lower case-hardening effect at the top of the material surfaces during drying, which would inhibit water removal (Fernandes and Rodrigues 2007). This treatment has been reported to improve the drying rate of the convective drying of plant-based foods (Kowalski and Rybicki 2017). The studies of ultrasound as a pretreatment for drying processes have been reported in many types of foods (de la Fuente-Blanco et al. 2006; Gamboa-Santos et al. 2014; Garcia-Perez et al. 2007; Santacatalina et al. 2015; Schossler, Jager, and Knorr 2012). In herbs, ultrasonic-treated dried parsley showed higher total phenolic, chlorophyll, and lutein content in comparison with non-treated leaves (Dadan et al. 2018). However, it was reported that the best method for pretreating parsley was steam blanching, considering the content of polyphenols, antioxidant activity, chlorophyll *a*, chlorophyll *b* and lutein. The effect of high-power ultrasound (HPU) as a pretreatment for the supercritical carbon dioxide drying process ($scCO_2$) of coriander leaves was reported (Michelino et al. 2018). The HPU pretreatment was used to improve the drying time and provided better microorganism inactivation effect in comparison with the non-treated dried product. Similar results were observed in the drying of thyme leaves (Rodriguez, Mulet, and Bon 2014), where the ultrasound treatment resulted in the reduction of drying time by 30% in comparison with untreated leaves. However, the decreasing drying time effect of ultrasound pretreatment was only observed at a drying temperature below 70 °C (drying temperatures of 40, 50, 60, 70 and 80 °C were investigated).

Herbs drying methods

Drying method is one of the main factors affecting the quality of dried herbs (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b) and its influence has been extensively studied. Table 3 summarizes studies reviewed in this section on the effect of drying methods on the quality of dried herbs. Drying methods applying high temperature would significantly decrease the amount of aroma compounds, since aroma compounds are heat-sensitive substances and can be evaporated from plant tissues easily during drying (Khangholil and Rezaeinodehi 2008). In contrast, the essential oil content in some types of herbs has been reported to be unaffected by the drying method tested, namely Mexican oregano (shade, sun, and 40 °C were compared) (Calvo-Irabien et al. 2009) and bay leaf (convective drying at 40, 50, and 60 °C, sun drying, and shade drying were compared) (Demir et al. 2004).

There are several well-known herb-drying methods such as sun drying, shade drying, freeze drying and hot-air drying. Among these drying methods, hot-air oven drying in the temperature range of 40–60 °C is the most common drying method used in herb drying studies in lab scale experiments (Shaw et al. 2016). Due to undesirable effects of high drying temperature on the quality of dried products, many studies have focused on the development of alternative drying methods, which could provide advantages over conventional methods. Some of these methods, such as solar-assisted drying (Ceylan, and Gurel 2016), microwave drying (Arslan and Özcan 2012), microwave-vacuum drying (Giri and Prasad 2007), infrared-assisted drying (Łechtańska, Szadzińska, and Kowalski 2015), heat-pump drying (Fatouh et al. 2006), and contact drying (Tarhan et al. 2011) are already being used in the industry. In the next sections, the effect of both conventional and newly developed drying methods on the quality of dried herbs will be reviewed.

Sun drying

Sun or solar drying is the oldest drying method that has been and is still used to dry many types of agricultural products, such as medical plants and aromatic herbs in most tropical or sub-tropical countries (Orphanides, Goulas, and Gekas 2016). During the process, fresh herbs are placed on well-ventilated drying racks and are exposed directly to the sunlight (Janjai, and Bala 2012). Sun drying may not be a suitable drying method for some types of herbs due to lower product quality. Sun drying causes a substantial color and aroma degradation in dried herbs. In the case of roman chamomile, the amount of major volatile components such as isobutyl isobutyrate, 3-methylbutyl isobutyrate and propyl

Table 3. Effects of drying methods on the quality of dried herbs.

Drying methods	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive compounds
Sun drying	Caused substantial color degradation in many types of herbs such as basil, parsley, coriander and thyme	No data available	Decreased essential oil content compared to hot air and shade drying in roman chamomile, basil, and lemon grass	Caused major degradation of aroma compounds in roman chamomile	Increased shrinkage compared to shade drying in *Vernonia amygdalina*	Decreased content of antioxidant compounds in *Mentha x piperita L.*
Shade drying	Better at preserving color of many types of dried herbs such as rosemary, thyme, mint, and sage dried with sun drying, how-air drying, microwave drying, and freeze drying	Good retention of chlorophyll content in *Mentha × piperita L.* and Origanum vulgare	Better preservation compared to sun drying in many types of herbs such as rosemary, mint, and sage	Preserving most aroma compound components of thyme similar to low temperature hot-air drying (50 °C) and sun drying	Better at preserving trichome structure of *Lippia Citriodora* compared to hot-air and vacuum drying	Showed good preservation of bioactive compounds in *Orthosiphon aristatus*, lemon balm, peppermint, and rosemary
Solar-assisted drying	No data available	No data available	Preserved more essential oil content in chamomile compared to sun drying	No data available	Better preservation of the structure of *Orthosiphon aristatus* compared to hot-air drying	Better preservation of bioactive compounds of *Orthosiphon aristatus* compared to hot-air drying
Hot-air drying	Caused substantial color degradation in many types of herbs such as basil, parsley, coriander and thyme especially with drying temperature higher than 60 °C	Caused major chlorophyll degradation in many types of herbs such as coriander, basil, and parsley	Decreased essential oil amount in most herbs especially with drying temperature higher than 60 °C	Caused major degradation of aroma compounds especially with drying temperature higher than 60 °C	Caused major degradation of herbs structures, especially with drying temperature higher than 60 °C	Caused major loss of bioactive compounds especially with drying temperature higher than 60 °C
Freeze drying	Excellent at preserving color of many types of herbs such as basil, coriander, bay leaf, rosemary, and thyme	Caused minor loss in chlorophyll content in basil	Better preservation of essential oil content in many types of herbs compared to most other drying methods	Caused the loss of major aroma compounds in parsley	Preservation of the structure of many types of herbs such as *Andrographis paniculate, Lippia citriodora, Ocimum basilicum L. and Orthosiphon aristatus* compared to other drying methods	Excellent at preserving bioactive compounds in many types of herbs such as thyme and spearmint. Caused major loss of bioactive compounds in *Lamiaceae* herbs including rosemary, oregano, marjoram, sage, basil, and thyme
Microwave Drying	Better preservation of color in many types of herbs such as parsley, basil, and rosemary compared to hot-air drying	Caused lesser loss in the chlorophyll content of many types of herbs such as parsley, basil, and coriander compared to hot-air drying	Better preservation of essential oil content in basil and coriander compared to hot-air drying	Good preservation of aroma compounds in many types of herbs such as coriander and basil	No data available	Better preservation of bioactive compounds of peppermint and spearmint compared to hot-air drying
Microwave-vacuum drying	Better preservation of color in mint compared to hot-air drying	No data available	Caused higher loss of essential oil content than hot-air drying in dried rosemary	Caused higher loss of some volatile compounds in rosemary compared to hot-air drying	Better preservation of structures of dried mint compared to hot-air drying	Better preservation of thymol content in *L. berlandieri* compared to hot-air drying and microwave drying
Heat-pump-assisted drying	No data available	No data available	No data available	No data available	Better preservation of structure of misai kucing and *Andrographis*	Better preservation of bioactive compounds of misai kucing, java

(*continued*)

Table 3. Continued.

Drying methods	Color	Chlorophyll content	Essential oil content	Aroma compound profile	Structural properties	Bioactive compounds
					paniculata compared to hot-air drying and solar-assisted drying	tea and *Andrographis paniculata* compared to hot-air drying and solar-assisted drying
Infrared drying	Caused substantially higher color degradation compared to other drying methods	Caused more loss of chlorophyll content compared to other drying methods	Caused higher loss of essential oil content in peppermint than hot-air drying but lesser loss in bay leaves and parsley	Showed good preservation of aroma compounds in peppermint and parsley compared to hot-air drying	No data available	Showed major loss in bioactive compounds of parsley; good preservation in peppermint
Fluidized bed drying	Good color retention in basil	No data available	No data available	No data available	No data available	Good preservation of bioactive compounds in basil
Supercritical CO_2 drying ($scCO_2$)	Better at preserving color of dried basil compared to hot-air drying	No data available	No data available	No data available	Better preservation of structures of dried basil compared to hot-air drying but worse in comparison with freeze-drying	Better preservation of bioactive compounds of dried basil compared to hot-air drying but worse in comparison with freeze-drying
Radio-frequency drying	Caused major color degradation of dried dill	Caused more degradation of chlorophyll content of dill compared to hot-air drying	No data available	No data available	No data available	Caused more degradation of bioactive compounds of dried dill compared to hot-air drying

tiglate of sun-dried roman chamomile was lower than that of hot-air-dried samples (dried at 40 °C) (Omidbaigi, Sefidkon, and Kazemi 2004). In the case of lemon grass, the sun-dried lemon grass was found to contain lower amounts of total essential oil in comparison with dried lemon grass obtained from hot-air drying (Hanaa et al. 2012). In basil (*Ocimum basilicum L.*), sun drying caused a greater reduction of essential oil content compared to shade drying and hot-air drying at 40 °C (Hassanpouraghdam et al. 2010). Sun drying also caused higher damage to the epidermal surface, shrinkage of the glandular trichomes and higher reduction of mineral content in *Vernonia amygdalina* leaves in comparison with shade drying (Alara et al. 2018).

Shade drying

Shade drying is another herb drying method that utilizes solar energy as a heating source. The process is conducted in almost the same way as sun drying, except that the herbs are placed under the shade in a room with good ventilation, low humidity (e.g. 22–27% for *Lippia citriodora* (Ebadi et al. 2015) and with no direct exposure to sunlight. During the shade-drying process, the ventilated air is heated up using solar energy before passing through the herbs (Sharma, Chen, and Lan 2009). This drying method could provide advantages over sun drying due to its ability to preserve light-sensitive substances and minimize light-induced chemical reactions such as oxidation. However, the drying time of shade drying is longer than sun drying, which is already considered to be an excessively long time process (Pirbalouti, Mahdad, and Craker 2013). Studies using this drying method have shown that shade drying is a better drying method in terms of preserving essential oil content and color of the dried products in comparison with other drying methods such as hot-air drying, sun drying, microwave drying and freeze drying for many types of herbs, namely rosemary (compared to oven drying at 45 °C and sun drying) (Khorshidi et al. 2009), *Tanacetum parthenium* (compared to oven drying at 40 °C and sun drying) (Omidbaigi, Kabudani, and Tabibzadeh 2007), thyme (compared to freeze drying) (Sárosi et al. 2013), basil (compared to oven drying at 40 and 60 °C and sun drying) (Hassanpouraghdam et al. 2010), mint (compared to convective drying at 40 °C) (Rababah et al. 2015), lemon balm (compared to convective drying at 40 °C) (Rababah et al. 2015), and sage (compared to convective drying at 40 °C) (Rababah et al. 2015). Also, shade drying is a better herb-drying process in terms of preserving the integrity of the trichomes. It was found that shade drying caused less damage to trichomes on dried *Lippia Citriodora* leaves in comparison with oven drying at 60 °C and vacuum drying at 40 °C (Ebadi et al. 2015).

In terms of bioactive compound content, shade drying also showed good retention of bioactive compounds in dried herbs such as misai kucing (*Orthosiphon aristatus*) (Abdullah, Shaari, and Azimi 2012). When shade drying, sun drying and air drying of misai kucing (40 °C) were compared, it was found that the shade-dried product showed the highest total phenolic content. In addition, shade drying was the only drying method that could maintain the rosmarinic acid content close to the fresh herbs. However, shade drying caused significant loss of the functional properties in some types of herbs, for example, the total antioxidant activity (TAA) of peppermint and lemon balm decreased significantly after shade drying (with a drying temperature of 25–32 °C for 10 days) and the loss of ascorbic acid and carotenoids in the dried samples was observed (Capecka, Mareczek, and Leja 2005). In addition, lower contents of aroma compounds of some shade-dried herbs were reported in comparison with other drying methods. In the case of thyme, shade-dried thyme showed lower essential oil content in the dried product compared to hot-air drying at 50 and 70 °C, sun drying and freeze drying (Rahimmalek and Goli 2013). Nevertheless, like sun drying, shade drying is still popular in rural areas or in small businesses due to its low investment cost and high-quality dried products (Janjai and Bala 2012).

Solar-assisted drying

Solar-assisted drying is a development of a well-known drying method, sun drying. Since solar energy is costless, the development of new solar-assisted drying techniques has gained considerable attention from researchers. This development is aimed at increasing the energy efficiency of the drying process and overcoming the major problems of traditional sun drying. Solar drying can be categorized into three main groups, (1) direct sunlight drying (which is the same as sun drying in this review), (2) indirect solar drying or convective solar drying, (3) mixed-mode or hybrid solar drying (Rabha, Muthukumar, and Somayaji 2017). Several studies on the development of solar-assisted dryers of herbs have been conducted in recent years, namely forced convection solar tunnel dryers (Rabha, Muthukumar, and Somayaji 2017), forced convection solar greenhouse dryers (Morad et al. 2017), solar-assisted fluidized bed dryers (Ceylan and Gurel 2016), and solar collector dryers (Sevik 2014). Many types of herbs dried using solar-assisted dryers have been studied, for example thyme and mint (indirect mode forced convection solar dryer) (El-Sebaii, and Shalaby 2013), peppermint (using solar tunnel greenhouse dryer) (Morad et al. 2017), java tea (solar greenhouse dryer with integrated heat pump) (Tham et al. 2017), parsley (solar-heat pump dryer) (Sevik 2014), rosemary (solar collector with auxiliary heater, at 50–80 °C) (Mghazli et al. 2017), saffron (heat-pump-assisted hybrid photovoltaic-thermal solar dryer) (Mortezapour et al. 2012), and misai kucing (solar-assisted heat pump dryer) (Gan et al. 2017). The solar tunnel greenhouse dryer for peppermint leaves has shown a reduction in drying time of 23–25% in comparison with a regular greenhouse dryer (Morad et al. 2017). The solar-assisted dryer using the combination of the solar collector and heat pump system, which can be used to create a nonstop working solar dryer, was used to obtain dried mint leaves with good quality (considering thermal damage, shrinkage, and taste), similar to regular sun-dried products (Sevik 2014). With the combination of solar collector, heat exchanger, reflector, main and secondary drying chambers, and supplementary water heater, the solar dryer for the drying of chamomile showed reduced drying time by 50% compared to direct sun drying. Additionally, the product had higher volatile oil content (Amer, Gottschalk, and Hossain 2018). The bin-type solar dryer integrated with a solar collector produced better-quality dried rosella flower and lemongrass compared to a regular solar dryer (Janjai, and Tung 2005). The integration of solar-assisted dryer and dehumidification system provided better color of dried pegaga leaves due to the lower drying temperature and relative humidity of the solar-assisted dehumidification drying system in comparison with a regular solar dryer (Yahya et al. 2004). Many of these new developments in solar drying showed considerable improvement in comparison to the traditional sun drying, especially in the energy efficiency of the process and quality of the dried products. However, the studies on the effect of these processes on aroma and color of dried culinary herbs is still lacking.

Hot-air drying

As mentioned above, solar-powered drying methods have the major drawback of excessively long drying times. In the industry, the most common and popular herb-drying method is oven drying (also called “convective drying” or “hot-air drying”), especially in non-tropical countries where sunlight is not sufficient for sun and shade drying (Orphanides, Goulas, and Gekas 2016). The major advantage of hot-air drying is the controllability of the process, in which food producers have full control over the process parameters such as drying temperature, drying time, and air velocity. These parameters can be adjusted to achieve the desired product properties (Orphanides, Goulas, and Gekas 2016). The process parameters for many types of herbs have been investigated and optimized for better quality of dried products (Orphanides, Goulas, and Gekas 2016). However, after hot-air drying, low content of total volatile compounds is obtained (Chua et al. 2019). Hot-air drying could lead to major degradation of herb aroma and high drying temperature could lead to the degradation of pigments (Fennell et al. 2004). Therefore, low drying temperatures (35–50 °C) have been suggested for the preservation of heat-sensitive compounds in the dried products (Müller et al. 1989). During the drying process, the hot air flow through the materials promotes the evaporation of moisture and volatile compounds (Orphanides, Goulas, and Gekas 2016) and creates a suitable environment for oxidation reactions (Antal 2010). Other major drawbacks of hot-air drying are high shrinkage of the products and high energy consumption (Orphanides, Goulas, and Gekas 2016). In addition, as hot-air drying is one of the most energy-intensive food processing methods, efforts have focused on reducing the energy consumption, increasing the process efficiency, and reducing the drying time (Won, Min, and Lee 2015). In the section below, the effects of hot-air drying parameters on quality degradation of dried herbs will be reviewed.

Effect of air temperature and humidity on the quality of dried herbs

Drying of herbs is recommended to be conducted by hot-air drying at 40–60 °C (Shaw et al. 2016). However, these drying temperatures lead to undesirable changes in aroma of the culinary dried herbs (Antal et al. 2011). It has been reported that increasing the drying temperature from 40 to 60 °C resulted in lower content of total volatiles, less fresh-like aroma and increase in spiciness, hay-like, sweet, earthy, and woody flavors in dried basil leaves (Calin-Sanchez et al. 2012). Similar results were observed in many types of herbs, such as peppermint (increasing the drying temperatures from 30 to 70 °C) (Rohloff et al. 2005), kaffir lime leaves (from 50 to 70 °C) (Jirapakkul, Tinchan, and Chaiseri 2013), *Achillea fragrantissima* (from 35 to 45 °C) (Abaas, Hamzah, and Majeed 2013), and sage (from 30 to 60 °C) (Venskutonis 1997). Drying temperatures higher than 60 °C result in the loss of most volatile compounds in the dried products in many types of herbs (*Allium schoenoprasum L., Anethum graveolens L., Anthriscus cerefolium (L.) Hoffm., Artemisia dracunculus L., Coriandrum sativum L., Levisticum officinale Koch. Mentha spicata L., Origanum majorana L., Petroselinum crispum (Mill.) Nym. ex A. W. Hill, Salvia officinalis L., Satureja hortensis L.*, and *Thymus vulgaris L.*) (Deans, Svoboda, and Bartlett 1991).

Additionally, increasing the hot-air drying temperature induces many other undesirable changes in the dried products, such as collapse of tissues (Prothon, Ahrne, and Sjoholm 2003), loss of bioactive compounds (Tambunan and Yudistira 2001), and increased color alteration (Calín-Sánchez et al. 2013). In the case of *Moringa Oleifera*, the color of leaves dried at 40 °C was better preserved in comparison with that from leaves dried at 50 and 60 °C (Ali et al. 2014). Structurally, in the case of *Vernonia amygdalina*, drying the leaves at 60 °C caused significantly higher damage to the epidermal surfaces of the leaves, shrinkage of the trichomes and higher degree of cell wall deformation than drying at 40 and 50 °C (Alara, Abdurahman, and Olalere 2019). Increasing the drying temperature also reduced the antioxidant capacity in many types of herbs, namely rosemary (*Rosmarinus officinalis*), motherwort (*Leonurus cardiaca*), and peppermint (*Mentha piperita*) (drying temperatures of 40 and 70 °C were compared) (Yi and Wetzstein 2011), meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*) (total phenols, salicylates, and quercetin content were compared at the drying temperatures of 30 and 70 °C) (Harbourne et al. 2009). In contrast, some studies report that increasing the drying temperature resulted in higher amounts of certain aroma compounds. This was the case for lemon verbena, in which a higher concentration of the volatile compounds was obtained at the drying temperature of 50 °C in comparison with drying at 30 and 40 °C (Shahhoseini et al. 2013). A similar result was observed in thyme leaves dried with hot-air drying at 30, 38 and 45 °C (Piga et al. 2007). The positive effect of increasing the drying temperature was also observed for the phytochemical content in the drying of herbal tea (containing several types of herbs including *Centella asiatica*, *Mentha arvensis*, and *Polygonum minus*), showing that the phytochemical content (including chlorophyll, ascorbic acid, niacin, riboflavin, and carotenoids) of the dried tea obtained at 70 °C was higher than that of tea dried at 50 °C (Mahanom, Azizah, and Dzulkifly 1999).

Freeze drying

Freeze drying has been suggested by several studies as a suitable drying method for preserving the fresh-like aroma of herbs due to its low operating temperature (Antal 2010). This drying process has been extensively reported to produce dried herbs with better aroma compared to other drying methods in many types of herbs such as spearmint, which showed less aroma compound reduction compared to hot-air dried leaves (Antal et al. 2011). Similar results were reported in basil leaves when freeze drying was compared to air drying at 50 °C (Di Cesare et al. 2003). Freeze drying showed better preservation of the yield and the chemical composition of the essential oil of purple and green basil leaves in comparison with sun drying, shade drying, hot-air drying at 40 and 60 °C, and microwave drying at 500 and 700 W (Pirbalouti, Mahdad, and Craker 2013). Similar results were shown for Iranian coriander (Pirbalouti, Salehi, and Craker 2017). Freeze-dried thyme resulted in only a 1–3% reduction in the total volatiles content (Venskutonis, Poll, and Larsen 1996). Freeze-dried oregano showed better color retention in comparison with air and vacuum-microwave drying (Yousif et al. 2000), and freeze-dried *Andrographis paniculata* leaves showed less shrinkage and higher porosity in comparison with hot-air drying (Tummanichanont, Phoungchandang, and Srzednicki 2017). However, comparing to microwave drying, freeze drying was reported to produce lower-quality dried products. In the case of garden thyme (*Thymus daenensis*), the freeze-dried leaves contained high amounts of essential oils and had good color, yet presented less intense aroma than leaves dried using microwave drying (Rahimmalek and Goli 2013). Similar results were observed in basil, for which freeze-dried leaves had lower contents of characteristic volatile compounds than microwave-dried leaves (eucalyptol, linalool, eugenol, and methyl eugenol content were compared). It was also found in the same study that freeze drying caused a greater reduction in chlorophyll pigments of the dried products in comparison with microwave drying (Di Cesare et al. 2003). Freeze drying could cause a major loss in the aroma compounds of dried herbs (Calín-Sánchez et al. 2013). It has been reported that freeze drying of parsley caused the loss of major volatile components such as p-mentha-1,3,8-triene and apiole (Diaz-Maroto, Perez-Coello, and Cabezudo 2002a). Loss of aroma was also observed in freeze-dried sweet basil (*Ocimum basilicum L.*) using a sensorial panel and, considering the high investment cost of the freeze-drying process, hot-air drying was suggested as the best drying method for drying of sweet basil (Díaz-Maroto et al. 2004). Similar results were observed in bay leaf (Diaz-Maroto, Perez-Coello, and Cabezudo 2002b).

Freeze drying produces high-quality dried herbs in terms of bioactive compounds in many types of herbs. When freeze-dried and hot-air-dried thyme leaves (*Thymus vulgaris*) were compared, freeze-dried thyme leaves showed higher yields of thymol compared to those obtained using oven drying at 30-50 °C and shade drying (Sárosi et al. 2013). Similar results were observed in freeze-dried rosemary leaves in terms of antioxidants compared to hot-air drying at 45 °C (Ibanez et al. 1999) and in freeze-dried spearmint leaves compared to sun drying, shade drying, convective drying and microwave drying (Orphanides, Goulas, and Gekas 2013). In contrast, freeze drying caused higher losses of bioactive compounds in *Lamiaceae* herbs including rosemary, oregano, marjoram, sage, basil, and thyme (hot-air, vacuum, and freeze drying were compared). In the study, freeze-dried samples had lower total contents of phenolic compounds, rosmarinic acid, and antioxidant capacity compared to the other drying methods (Hossain et al. 2010).

Microwave drying

Microwave drying is a drying technique which is currently available in herb processing industry (Moses et al. 2014; Wray and Ramaswamy 2015). It allows rapid evaporation of water from food, providing relatively shorter drying times compared to many drying methods (convective drying, shade and sun drying, freeze drying) (Chi et al. 2003) and decreased energy consumption in the drying process (Di Cesare et al. 2003). Microwave-dried products showed less shrinkage, better color and rehydration capacity compared to hot-air drying (Kathirvel et al. 2006). The quality of microwave-dried products is influenced by drying parameters such as microwave power (W), drying time, the initial moisture content of the product, and the dielectric properties of the materials (Moses et al. 2014). Increasing the microwave power from 360 to 900 W reduced the drying time of parsley by 64% and microwave-dried parsley showed good color retention with only slightly darker color than fresh parsley (Soysal 2004). Similar results were observed in coriander (Sarimeseli 2011), where increasing the microwave power from 180 to 360 W resulted in an increasing diffusivity coefficient while the rehydration capacity of the dried coriander leaves decreased.

In comparative studies, the quality of microwave-dried herbs was higher than that obtained with other drying methods. Microwave-dried basil leaves showed higher retention of volatiles compared to the dried products from convective drying (50 °C) and freeze drying. In the study, the microwave-dried leaves showed fewer color changes in the dried product compared to the convective-dried product. The lower color alteration could be the result of the shorter drying time of microwave drying (Di Cesare et al. 2003). A comparison study of microwave drying (with the microwave power of 700 W, 2450 MHz), sun drying, and hot-air drying (at 50 °C) of rosemary leaves showed that the color of microwave-dried rosemary was better than that of hot-air-dried products (Arslan and Özcan 2008). A similar result was observed in microwave-dried coriander foliage dried at the microwave power of 295 W, which showed better color retention in comparison with the convective dried sample at 50 °C.

Microwave drying provides high-quality dried products in terms of preserving or enhancing the content of bioactive compounds. Applying microwave drying at 850 W resulted in higher intactness of trans-β-carotene and higher extractability of pigments in dried coriander leaves compared to convective drying at 45 °C (Divya, Puthusseri, and Neelwarne 2012). Similar results were observed in microwave-dried sage leaves at the microwave power of 850 W (Hamrouni-Sellami et al. 2013), in which the microwave-dried product showed higher retention of total phenolic compounds, flavonoid content and antioxidant activity in comparison with the dried products dried using convective drying at 45 °C. Similar results were observed in *Gynura pseudochina* leaves (Sukadeetad et al. 2018).

Microwaves can be used in combination with other drying methods such as hot-air drying either as a pre-drying stage to reduce the initial moisture content of the materials or as the final stage of drying (Orphanides, Goulas, and Gekas 2016). However, the major drawback of microwave drying is the non-uniformity in heating, which results in the formation of temperature gradients in the product, especially in the large-size products, during the drying process. This non-uniform heating could lead to non-uniform dehydration of the product, overheating, and quality degradation (Ozkan, Akbudak, and Akbudak 2007). Nevertheless, the interest in microwave drying of herbs has increased in recent years. This is likely because herbs are usually smaller and thinner in size than most other solid foods, and thus non-uniform heating might not be a major drawback for microwave drying of herbs. However, microwave drying for some types of herbs, such as marjoram (Raghavan et al. 1997) and rosemary (Rao et al. 1998), were reported to cause a greater reduction of aroma compounds in comparison with many drying methods, including convective drying, shade drying, and sun drying. Microwave drying time is much faster than all the compared conventional drying methods. However, for some types of herbs, further studies of microwave drying parameters are needed to optimize the process and improve the quality of the dried products (Moses et al. 2014).

Microwave-vacuum drying

The combination of microwave and vacuum drying has recently gained attention (Orphanides, Goulas, and Gekas 2016). The process is conducted by using microwave irradiation as the heating source to increase the temperature of the food materials in the sub-atmospheric pressure drying chamber. The vacuum creates the driving force of water evaporation, resulting in faster drying rates in comparison with convective drying and microwave drying (Soysal 2004). Compared to hot-air drying, microwave-vacuum drying could reduce the drying time by 70–90% and also produce better-quality products (Giri and Prasad 2007). The level of thymol in vacuum-microwave dried *L. berlandieri* was 1.3

times higher than those dried using air drying (Yousif et al. 2000). The microwave-vacuum drying of mint leaves resulted in better color retention of dried products compared to hot-air drying. SEM images of these microwave-vacuum dried products showed more porosity and less collapse compared to hot-air-dried samples (Therdthai and Zhou 2009). However, the major limitation of the vacuum-drying process is the capacity of the vacuum pump. With the high load of initial moisture from food materials, the vacuum pump may exceed its capacity quickly, resulting in a less efficient process.

In contrast, microwave-vacuum drying has been reported to reduce the dried product quality in some types of herbs, such as rosemary. Microwave-vacuum drying resulted in a higher loss of volatile compounds of dried rosemary in comparison with hot-air drying, and with the combination of hot-air drying and microwave-vacuum drying (Szumny et al. 2010). The microwave-vacuum-dried rosemary contained fewer volatile compounds and lower sensory quality in comparison with hot-air drying at 60 °C. In their study, the authors suggested that microwave-vacuum drying was a "not suitable" drying method for rosemary. However, in the same study, the combination of hot-air drying and microwave-vacuum drying (called convective pre-drying and vacuum-microwave finish-drying (CPD-VMFD) was reported to provide the highest concentration of volatile compounds in the dried products. To sum up, microwave-vacuum drying is a promising combined drying method with good potential to become a suitable method for drying herbs. However, further optimization studies need to be done.

Heat-pump-assisted drying

Heat-pump drying is another drying technique development aimed at increasing the efficiency of traditional convective drying. A heat pump is usually coupled with another air-drying unit to increase the initial input air temperature. The system could be called "heat pump dryer or heat pump-assisted dryer" (Fatouh et al. 2006). The heat pump dryer is suitable for industrial herb drying as it can be operated in wide ranges of air velocity and drying temperatures (Fatouh et al. 2006). Another major benefit of heat pump dryers is their ability to dehumidify the outlet air of the drying unit. The dehumidifying effect occurs when the temperature of the evaporator is lower than the dew point of the air at the evaporator inlet (Fatouh et al. 2006). Heat-pump drying could provide better-quality dried products due to its ability to control the properties of the air during the process. Heat-pump solar drying of java tea (*Orthosiphon aristatus*) showed better controllability of the relative humidity of the drying room in comparison with regular solar greenhouse dryers, especially during the nighttime. The dehumidifying system reduced the relative humidity of the drying room by 10–15% and was able to maintain the maximum relative humidity of 65%. Moreover, the drying rate of the heat-pump-integrated solar greenhouse was 3-4 times better than that of a regular greenhouse dryer (Tham et al. 2017).

The focus of recent studies has been the heat-pump drying of medical herbs, in which the content of bioactive compounds in the dried products has been investigated (Gan et al. 2017; Klungboonkrong, Phoungchandang, and Lamsal 2018; Tummanichanont, Phoungchandang, and Srzednicki 2017). Most of the studies reported that heat-pump dryers produced better-quality dried products in terms of preserving bioactive compounds in comparison with other drying methods, such as in the case of misai kucing (compared with solar drying) (Torki-Harchegani et al. 2017) and *Andrographis paniculata* (compared with hot-air drying, microwave drying and freeze drying) (Tummanichanont, Phoungchandang, and Srzednicki 2017). When the effect of heat-pump drying on the quality of *Andrographis paniculata* was investigated, it was found that the heat-pump drying (with dehumidifier function, called heat pump dehumidifier dryer (HPD)) at 40, 50 and 60 °C resulted in higher amounts of bioactive compounds, including andrographolide, neoandrographolide and total phenolics, in comparison with hot-air-dried samples at the same drying temperatures. In the same study, heat-pump drying was shown to be better at maintaining the original shape of parenchyma cell structures of the dried products compared to air drying. A comparison study on the effect of heat-pump drying (using heat pump dehumidify dryer; HPD), convective drying and freeze drying on the quality of java tea, found that heat-pump-dried java tea at the drying temperature of 60 °C showed good retention of total phenolic content and antioxidant activity of the dried products similar to freeze-dried products (Klungboonkrong, Phoungchandang, and Lamsal 2018). Additionally, the HPD system reduced the drying time by 44.8% compared to convective drying at the same drying temperature. Moreover, the microstructure of the products dried using HPD showed fuller and more regular cell structure than the convective dried product. Overall, heat-pump drying provided promising results for improving the content of bioactive compounds and the structural properties of the dried products. However, further studies of this drying technique in culinary herbs are needed to test the effect of the process on their aroma.

Infrared drying

The major advantages of this drying process are the adaptability, simplicity, fast heating rate, and fast drying rate (Ashtiani, Salarikia, and Golzarian 2017). During the process, the electromagnetic energy from infrared wavelength radiation is transmitted and absorbed by the material generating heat from inside of the materials due to the changes of molecular vibrational state (Krishnamurthy et al. 2008). Infrared drying has higher energy efficiency compared to hot-air drying. However, only a few studies of herb drying using infrared have been conducted in recent years. When drying mint leaves, the energy efficiency and drying rate of infrared drying were higher than during convective drying (drying temperatures of 30, 40, 50 °C were compared) (Ashtiani, Salarikia, and Golzarian 2017). Increasing the infrared drying temperature resulted in higher crocin and safranal content of dried saffron (Torki-Harchegani et al. 2017). These compounds are the main chemical compounds contributing to dried saffron quality. Infrared irradiation is suitable for thin-layer drying due to its short traveling distance in the materials and the dependency of the contacted area on the materials. Moreover, the fast drying rate of infrared drying (compared to hot-air drying) (Ashtiani, Salarikia, and Golzarian 2017; Torki-Harchegani et al. 2017) and the ability to maintain high drying rate at lower moisture content (Pääkkönen, Havento, and Galambosi 1999) would make infrared drying a promising alternative drying method for herbs. However, Chua et al. (2019) reported that the non-uniform heating of infrared leads to the degradation of the aroma quality in dried herbs.

Fluidized bed drying

Fluidized bed drying has been implemented in the food industry for many types of agricultural products, including herbal leaves (Gangopadhyay and Chaudhuri 1979). The process is carried out by passing high-velocity hot air (high enough to create fluidization of the products) to the drying bed where the products are placed. The drying rate of this method is much higher than traditional convective drying due to the higher heating rate of the fluidization heating. For fluidized bed drying of lemon myrtle leaves, increasing the drying temperature (drying temperatures of 30, 40, and 50 °C were compared) resulted in higher retention of citral content (which contributes the "citrus" aroma) of the dried product (Buchaillot, Caffin, and Bhandari 2009). However, the lowest tested drying temperature (30 °C) showed better color retention, and the highest drying temperature (50 °C) showed unacceptable color quality degradation.

Herbs may not be suitable for fluidization drying due to their high moisture content, large surface area to volume ratio, and rough surfaces, which could lead to poor air percolation. To overcome this problem, vibrofluidized bed drying has been developed (de Aquino Brito Lima-Corrêa et al. 2017). The vibrofluidized drying process is a type of fluidized bed dryer, which is attached with a vibrator module to enhance the performance of the fluidized bed dryer. Vibrofluidized bed drying achieved the requirement of moisture reduction and moisture homogeneity of dried basil leaves while conventional fluidized bed drying did not. However, the loss of eugenol content of the dried product was observed with drying temperatures of 45 and 60 °C.

Supercritical CO_2 drying ($scCO_2$)

This process uses supercritical carbon dioxide as a drying medium. The major advantages of this drying technique are mild operating temperature (usually close to ambient temperature), low or non-presence of oxygen, low product shrinkage, and better rehydration capacity of dried products. Only a few studies of $scCO_2$ drying of herbs have been conducted. CO_2 drying of basil was reported (Busic et al. 2014) in comparison with other drying techniques including convective drying (40 °C for 26 h) and freeze drying (−20 °C at 0.005 bar for 4 days). The results showed that the best quality of dried basil was achieved by freeze drying, followed by $scCO_2$ drying, while convective drying showed the worst dried product quality considering the preservation of color, bioactive compounds, and the fresh-like characteristic properties. However, it was suggested that $scCO_2$ drying was the most suitable drying process among the three studied drying methods due to the acceptable quality of the dried herbs, and drastically shorter drying time (2–3 h) compared to freeze drying (4 days) and air drying (26 h). Another study of $scCO_2$ drying of herbs was conducted in combination with ultrasound pretreatment in coriander leaves (Michelino et al. 2018). The results showed that $scCO_2$ drying provided good inactivation of microorganisms. According to the results, yeast, molds and mesophilic bacteria were reduced by 4 Log during the drying process. However, the analysis of sensory and chemical properties of dried products was not reported in the study.

Radio-frequency drying

Radio-frequency (RF) drying combines the utilization of radio frequency heating and convective drying. Radio frequency heating relies on the dielectric properties of the food materials, similar to microwave heating, but with differences in wave frequencies (Nijhuis et al. 1998). Radio-frequency heating could help increase the drying rate, especially during the falling rate period where the conventional convective drying encounters its limitation (Thomas 1996). To the best of our knowledge, there is only one RF drying study of herbs. The effect of RF drying with infrared was compared with convective drying on the quality of dill greens (Naidu et al. 2016). RF drying showed faster drying rates than convective drying at 50 °C. However, the RF-dried dill greens showed the lowest bioactive compound content (including chlorophylls *a* and *b*, carotenoids and ascorbic acid) in comparison with the dried products from convective drying (50 °C, with 58–63% RH and 28–30% RH) and infrared drying. According to the results, RF drying might not be a suitable drying method for herbs considering the degradation of chlorophyll and resulting color changes.

Hybrid drying methods

Hybrid drying methods are the combination of two or more drying techniques to overcome the problem of single stage drying. In this paper, we have reviewed heat pump drying, solar assisted drying, microwave-vacuum drying, and radio-frequency drying. These drying techniques have recently gained attention from researchers due to their ability to shorten processing time, minimize quality degradation and maintain the process efficiency (Chou, and Chua 2001). Currently, the three methods that have received the most attention are probably solar-assisted drying, microwave-assisted drying, and heat pump-assisted drying (Chou, and Chua 2001; Jin et al. 2018). However, the information on the effects of these hybrid technologies on the quality of dried herbs is limited.

Conclusions

Improving the quality characteristics of dried herbs has been the main subject of many studies on drying and pre-drying methods for the past 20 years. A number of pre-drying treatments and drying methods, investigated in different herbs, have been developed, showing an improvement in quality, better energy conservation, and better process efficiency. Hybrid-drying techniques have shown promising results on the improvement of dried herbs quality including both color and aroma. In spite of these technological developments, obtaining high-quality dried herbs is still an issue as herbs are highly sensitive to different pre-drying and drying process conditions, mainly in regard to color and aroma. Moreover, the quality of dried herbs is very sensitive to the type of herb, harvesting season, postharvest practices, age of the plant and storage conditions. Therefore, optimization of quality requires studying each specific pre-drying and drying method for each type of herb.

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No potential conflict of interest was reported by the authors.

Author contributions

G. Thamkaew wrote the manuscript, created figures and tables. I. Sjöholm and F. Gómez Galindo supervised and revised the manuscript.

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Paper II

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Influence of pulsed and moderate electric field protocols on the reversible permeabilization and drying of Thai basil leaves

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ABSTRACT

The effect of electroporation parameters on the reversible permeabilization of cells in Thai basil leaves and, specifically, cells on the leaf surface was investigated, as electroporation of stomatal guard cells decreases drying times. Various combinations of PEF and MEF parameters were applied. The effect of these parameters on the electroporation of the leaf surface was assessed with microscopic observations and the electroporation of the bulk tissues was tested with electrical resistance measurements. With PEF and MEF, the electroporation of the epidermal cells increased with increasing treatment time. Compared to epidermal cells, guard cells required larger number of pulses to achieve homogeneous electroporation. Results showed that electroporation of cells on the leaf surface increased with the increase of voltage, pulse width and number of pulses. The electroporation of cells in the bulk tissues increased with increasing voltage and pulse width. Six specific combinations of parameters were found to electroporate the guard cells on the leaf surface while maintaining the viability of the leaves.

1. Introduction

Pulsed electric field (PEF) is a non-thermal food processing technique based on the electroporation of cell membranes in biological tissues resulting in an increase in cell permeability (Gómez Galindo, 2016). The effect of PEF has been studied in many types of foodstuffs, such as meat (Bhat, Morton, Mason, Jayawardena, & Bekhit, 2019; Gómez et al., 2019; Kantono et al., 2019), fruits (Gagneten, Leiva, Salvatori, Schebor, & Olaiz, 2019; Nierop Groot, Abee, & van Bokhorst-van de Veen, 2019; Nowacka et al., 2019), vegetables (Leong, Du, & Oey, 2018; Mannozzi et al., 2018; Putnik et al., 2018), and herbs (Bansal, Sharma, Ghanshyam, & Singla, 2014; Dobreva, Tintchev, Dzhurmanski, & Toepfl, 2013). Depending on the cell properties (i.e. size, shape and orientation) and electropulsation parameters (i.e. field strength, duration and number of pulses), the application of PEF may cause lethal damage to cells due to irreversible loss of cell membrane permeability properties and leakage of cytoplasmic contents. However, by strict control of the electropulsation parameters, permeabilization may evade affecting the cell viability as the cells recover from the disturbance provoked by the electric field (Bodenes et al., 2019; Gómez Galindo et al., 2009; Rajeckaite et al., 2018; Telfser & Gómez Galindo, 2019). In food processing, PEF is mostly used in its irreversible form to inactivate microorganisms (Evrendilek, Karatas, Uzuner, & Tanasov, 2019; Mahendran et al., 2019; Montanari et al., 2019), improve extraction yield (Gagneten et al., 2019; Jaeschke et al., 2019; Martinez et al., 2019), and enhance mass transfer in the dehydration process (Gómez et al., 2019; Huang et al., 2019; Lammerskitten et al., 2019; Wiktor & Witrowa-Rajchert, 2019).

The application of irreversible PEF as a pre-drying treatment has gained attention from researchers (Gómez et al., 2019; Kwao, Al-Hamimi, Damas, Rasmusson, & Gómez Galindo, 2016; Telfser & Gómez Galindo, 2019; J. Wang, Zhang, & Fang, 2019; Wiktor, Dadan, Nowacka, Rybak, & Witrowa-Rajchert, 2019) due to the resulting decrease in drying time, which is especially beneficial for the drying of heat-sensitive foods (Lebovka, Shynkaryk, & Vorobiev, 2007) such as apple, coconut, potatoes, and carrots (Ade-Omowaye, Angersbach, Taiwo, & Knorr, 2001). For herbs, the high energy input of irreversible electroporation drastically decreased the drying time of sweet basil with a concomitant degradation of aroma and color of the dried product. On the other hand, the application of reversible PEF as a pre-drying treatment could be applied to decrease drying time as well as improving color and aroma of the dried product. The conditions of the applied reversible electroporation are such that the stomatal guard cells are electroporated and remain open during the drying process (Kwao, Al-Hamimi, Damas, Rasmusson, & Gómez Galindo, 2016). Stomatal opening facilitates the transport of water vapor from the plant leaf to the environment (Taiz, Zeiger, Møller, & Murphy, 2015).

Cell permeabilization can also be achieved using moderate electric

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field (MEF). MEF involves a simpler, more direct application of electrical current (i.e. no capacitors, pulse forming networks, etc.) as an AC current (vs. DC in PEF) at considerably lower field strengths than PEF. The electroporation provoked by MEF can be either reversible or irreversible, depending on the strength of the electric field (Sensoy & Sastry, 2004), which can be modified by changing the frequency, field intensity or treatment time. MEF application may benefit the drying process due to the enhanced permeability of treated tissues (Kulshrestha & Sastry, 2010; W. C. Wang & Sastry, 1997). However, the effect of MEF on electroporation in the guard cells is currently unknown.

To the best of our knowledge, only a few studies on the effect of electrical parameters on the reversible electroporation of plant leaves (Dymek, Dejmek, & Gómez Galindo, 2013) and its consequences on drying (Kwao et al., 2016; Telfser & Gómez Galindo, 2019) are available. Kwao et al. (2016) uniformly and reversibly electroporated the epidermal cells of basil leaves prior to drying and showed that by increasing the pulse width and the pulse space, the guard cells could be electroporated. This finding suggested that the electroporation of stomatal guard cells may be achieved through specific combinations of PEF parameters. This paper details a systematic study where several combinations of PEF and MEF parameters were tested to identify specific conditions where both the homogeneous permeabilization of the leaf surface and guard cell electroporation occur. Electroporation was assessed using fluorescence microscopy, and electroporation of the bulk tissue was examined through the measurement of electrical resistance.

2. Materials and methods

2.1. Raw material

Potted Thai basil (*O. basilicum* var. thyrsiflora) was grown at the local grower's greenhouse (Kabbarp, Sweden) in a controlled environment for 28 days before being transported to our laboratory. The potted plants were placed under growing lamps with a light intensity of 200 μmol m^{-2} s^{-1} for 16 h./day under ambient temperature conditions to prevent starvation. All plants were kept in the above conditions for at least two days before the day of experiment and were used within five days after arriving from the grower. Leaves without damage in the range of 2 ± 0.2 cm in width and 3.5 ± 0.3 cm in length were harvested. Harvested leaves were kept in a closed plastic container with wet tissue before the experiment to prevent moisture loss. All leaves were used within 15 min after harvest.

2.2. Treatments

2.2.1. Electrical treatments

Three Thai basil leaves (0.5 g) were placed in an electroporation chamber with a 0.5 cm gap between electrodes. The chamber was filled with 50 ml of NaCl solution (130 μS/cm) which is sufficient to cover both the electrodes and the samples (ratio mass: solution of 1:100). The chamber was then connected to a pulse generator (ADITUS AB, Lund, Sweden) for PEF treatments or to an AC Power source (3000 VA, BK Precision, CA, USA) for MEF treatments. The temperature increment during PEF and MEF treatments were less than 2 °C. Electroporation protocols were delivered by combining the parameters in the range shown in Table 1. Treated samples were washed with distilled water and placed on absorbent paper to remove excess water before drying and microscopic analysis.

2.2.2. Drying

Thai basil leaf samples (were evenly spread in a wire mesh (stainless steel) tray with square holes of 5.0 × 5.0 mm^2. The mass of the material per area of the mesh was 0.08 kg/m^2. To prevent the leaves from being blown out of the tray, another mesh tray was placed on top of the first containing the leaves. The gap between the two trays was 1 mm. The drying experiment was performed at 40 °C and an air velocity of 2 m/s in a convection drying oven to a final water activity of 0.46 (MR = 0.05). The drying time was dependent on the treatment. The samples were treated with electroporation protocols with three different levels of surface permeabilization: 1) reversible permeabilization with electroporated guard cells, 2) reversible permeabilization without electroporated guard cells, 3) no permeabilization (control). According to these surface permeabilization levels, five electroporation protocols were selected for the drying experiment. The electroporation parameters of these protocols are listed in Table 2. PEF1 and PEF2 refer to two protocols where the tissue was reversibly permeabilized and the guard cells were electroporated, while PEF3 and MEF were protocols where the tissue was reversibly permeabilized without electroporating the guard cells. The control refers to untreated samples. Three drying procedures were performed per each experimental condition.

2.3. Analysis

2.3.1. Microscopic observation of leaf surface electroporation and viability of the tissue

To investigate the effect of the different protocols on the electroporation of the cells on the leaf surface, propidium iodide (PI) was used as an electroporation indicator. The method was described by Dymek et al. (2013), where PI (Sigma-Aldrich, USA, λ_{ex} = 535 nm, λ_{em} = 617 nm) was used to stain the nucleus of the cells upon permeabilization. Three Thai basil leaves were placed in the electroporation chamber filled with 250 μM PI solution in 10 μM PBS solution with a conductivity of 130 μS/cm before being subjected to electrical treatment. Treated leaves were rinsed with running tap water and gently patted dry with absorbent paper prior to microscopic examinations. The observation was conducted using a fluorescence microscope (Elipse Ti−U, Nikon, Japan) at 10× magnification. The images of the samples were taken with a digital camera (digital sight DS-Qi1Mc, Nikon Co., Japan).

Survival of the samples was investigated using fluorescein diacetate (FDA; Sigma-Aldrich, USA, λ_{ex} = 492 nm, λ_{em} = 517) as described by Dymek et al. (2013), which was used to stain viable cells. FDA stock solution (12 μM) in acetone was prepared and stored in the dark at 4 °C. On the day of the experiment, the stock solution was diluted with deionized water to the final concentration of 12 × 10^{-4} μM. Leaf samples were electroporated in NaCl solution with a conductivity of 130 μS/cm (adjusted with NaCl) using PEF or MEF protocols. After electroporation, three Thai basil leaves from each protocol were incubated in a closed container with wet paper towels at 4 °C for 20 h

Table 1
Range of parameters used for the studied electroporation protocols.

Protocol	Voltage (V/cm)	Pulse width (μs)	Pulse space (μs)	Number of pulses	Frequency (Hz)	Treatment time (ms)	Polarity
PEF	100–650	50–1000	760	0–1500	–	–	Monopolar
PEF	650	50, 175	760	0–500	–	–	Bipolar[a]
PEF	650	50, 175	380–1520	0–500	–	–	Monopolar
MEF	50–600	–	–	–	60–1200	0–25,000	Bipolar sinusoidal

[a] The total length of the pulse was divided into half positive and half negative.

Table 2
Parameters of the electroporation protocols for the drying tests.

Protocols	V/cm	Pulse width	Pulse space	Number of pulses	Frequency (Hz)	Treatment time (ms)	Guard cells electroporation	Specific energy input (kJ/kg)
PEF1	650	50	760	200	–	–	Yes	27.46
PEF2	650	175	760	125	–	–	Yes	60.07
PEF3	650	50	760	150	–	–	No	20.60
MEF	100	–	–	–	1200	1200	No	20.23
Control	–	–	–	–	–	–	No	0

before FDA staining. After incubation, samples were submerged in the diluted FDA solution in the dark at room temperature for 30 min. The samples were then rinsed with deionized water and examined under fluorescent microscopy. The survival of samples was determined by the occurrence of stained living cells on the leaf surface. All microscopic observation was performed on the bottom-side of the leaves to obtain clear observation of the stomata.

2.3.2. Electrical resistance

Changes in the electrical resistance of the leaves were evaluated as described by Dymek et al. (2013) with some modifications. Leaves were cut into 1 cm^2 square pieces with a sharp blade. The sampling area of the leaf is shown in Fig. 1. The leaf piece was placed between two flat stainless-steel electrodes with wet (130 μS/cm NaCl solution) filter paper (Qualitative filter paper, grade MN 713, Macherey-Nagel, Düren, Germany) making an electrodes-sample sandwich (Fig. 2). The electrodes were lightly squeezed with a metal clamp for good attachment between the sample and electrodes.

To evaluate the changes in electrical resistance provoked by PEF, the electrodes sandwich (Fig. 2) was connected to the PEF generator. Electrical resistance of the samples was measured using the CythorLab software supplied with the pulse generator (Version 1.2, build 20,041,215, ADITUS AB, Lund, Sweden) at a frequency of 100 Hz, 1 s before and 1 s after the treatments. The electrical resistance ratio (R_{ratio}) was calculated using following Eq. (1):

$$\text{Electrical resistance ratio;Rratio} = \frac{\text{Electrical resistance after pulse application}}{\text{Electrical resistance before pulses application}} \quad (1)$$

To evaluate the changes in electrical resistance provoked by MEF, the electrode sandwich was connected to the AC Power source and an LCR meter (4192 A LF Impedance Analyzer, Agilent Technologies

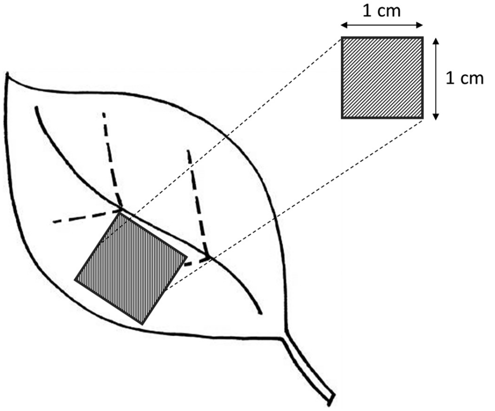

Fig. 1. Sampling of Thai basil leaves.

Sweden AB, Kista, Sweden). The schematic diagram of the setup is shown in Fig. 2. To measure the electrical resistance before and after pulse application, a three-way manual switch was connected between the generator and the LCR meter to allow quick switching between pulse application and electrical resistance measurement. The resistance measurement was done before the pulse and 5 s after pulse application at a frequency of 100 Hz. The R_{ratio} of the samples was calculated using Eq. (1).

2.3.3. Moisture content

The moisture content of the samples was determined by placing the samples in a hot air convection oven (AB Termo-Glas, Gothenburg, Sweden) at 105 °C for 24 h (AOAC, 2000). The analysis was performed in triplicate.

2.3.4. Water activity (aw)

After drying, the final water activity was measured with an Aqualab (Model CX-2, Decagon devices Inc., Pullman, WA) water activity analyzer at 20 °C. The analysis was done in triplicate.

2.3.5. Moisture ratio (MR)

The moisture ratio (MR) of the samples during drying was calculated using the Page model for moisture ratio (Erbay & Icier, 2010) according to Eq. 2. It is assumed that the equilibrium moisture content is negligible.

$$MR = \frac{Mt - Me}{Mo - Me} = \frac{Mt}{Mo} = \exp(-kt^n) \quad (2)$$

where MR is the dimensionless moisture ratio, M_t is the moisture content at any time (kg water/kg dry weight), M_e is the equilibrium moisture content (kg water/kg dry weight), M_0 is the initial moisture content (kg water/kg dry weight), k is the drying rate (min^{-1}), and t is the drying time (min).

The effective moisture diffusivity (D_{eff}, $m^2\,s^{-1}$) was calculated using the simplified Fick's law, (Sarimeseli, 2011) as follows:

$$MR = \frac{Mt}{M0} = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 \times D_{eff}}{4L^2} \times t\right) \quad (3)$$

where L is the half thickness of the leaves (m) and t is the drying time (s).

The diffusion equation is solved for an infinite slab, assuming that the initial moisture distribution is uniform throughout the material, mass transfer is symmetric with respect to the center, constant temperature and diffusivity coefficients, and negligible shrinkage and external resistance (Doymaz, 2006; Sarimeseli, 2011).

2.4. Statistical analysis

Statistical significance testing was performed using SPSS (v.25.0, IBM Corp., Armonk, NY, USA) at a significance level of 0.05. Post-Hoc tests were performed using the Tukey-HSD method. Curve fitting was performed using the MatLab curve fitting toolbox (Matlab R2019a, MathWorks, Inc., MA, USA). The coefficient of determination (R^2), the root means square error (RMSE), and the sum of square error (SSE) were used to evaluate the fitness of the model.

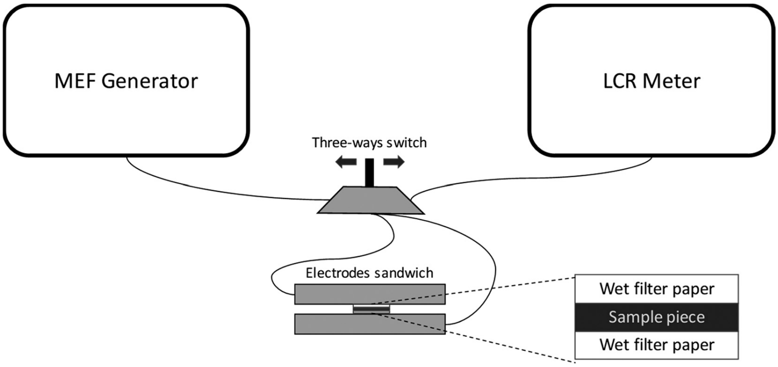

Fig. 2. Schematic diagram of MEF experiment setup.

3. Results

3.1. Microscopic observations

Microscopic observations of treated Thai basil leaves showed the permeabilization of the cells on the surface after electroporation. When homogeneous electroporation occurred, the nuclei of electroporated cells were able to be observed across the entire leaf surface as bright red circles (Fig. 3.A). Closer examination of the surface allowed the observation of non-electroporated and electroporated guard cells (Fig. 3.B and C). After the application of monopolar treatments, electroporated cells were observed only on the anode-facing side of the leaves (abaxial surface), where stomata are most abundant. The application of bipolar treatments (both PEF and MEF) induced the penetration of PI into cells on both sides of the leaves. However, to compare the results from the samples treated with monopolar and bipolar treatments, only the micrographs from the anode-facing side of the leaves were chosen.

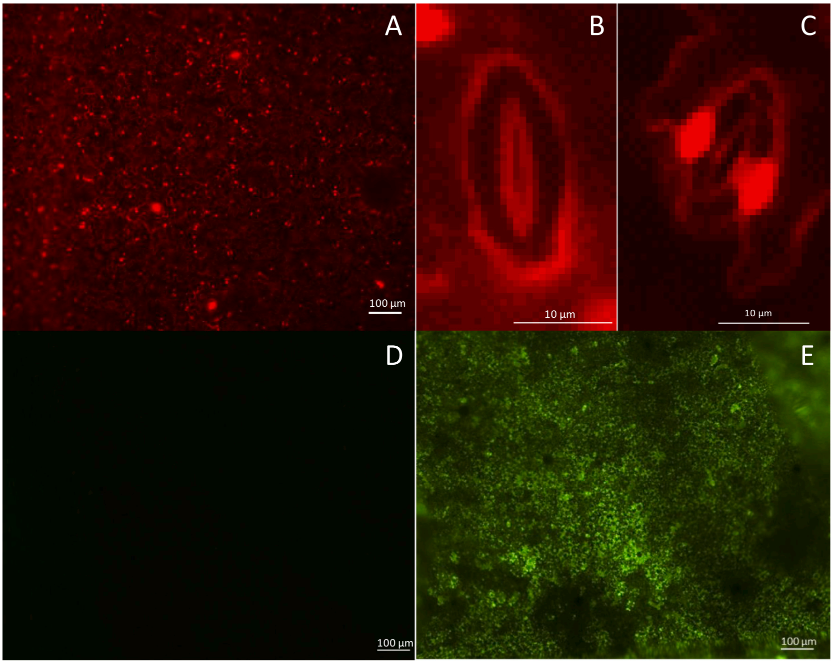

Fig. 3. Representative micrographs from microscopic observations of basil leaf surface. A: Homogeneous surface permeabilization detected by PI, B: Non-electroporated guard cells, C: Electroporated guard cells, D: Dead cells, and E: Viable cells.

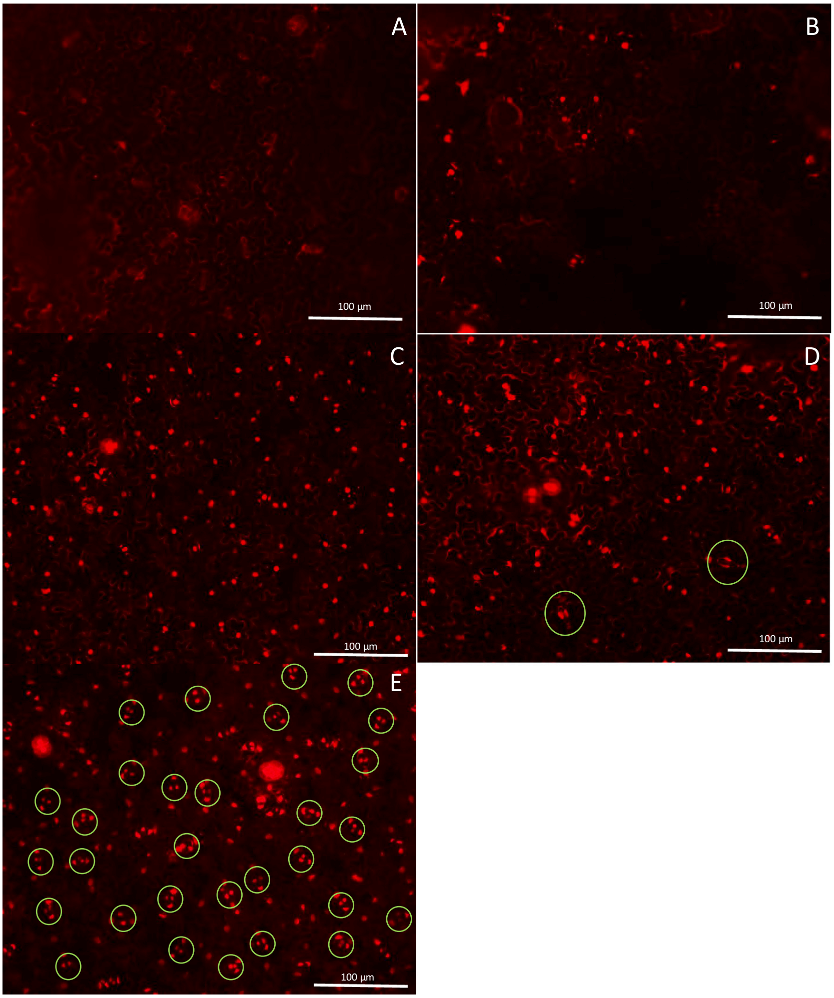

Fig. 4. Representative micrographs of PEF-treated Thai basil leaf samples showing the permeabilization progression of cells on the leaf surface. All samples were treated with monopolar pulses of 650 V/cm and a pulse width of 50 µs at a differing number of pulses. A: untreated sample, B: 25 pulses, C: 100 pulses, D: 150 pulses, and E: 200 pulses. Green circles in D and E indicate the electroporated guard cells.

The viability of the tissue was determined by the micrographs of the samples after the FDA test. Since dead cells cannot hold the FDA molecules, they do not show fluorescence (Fig. 3.D), while the living cells appeared as bright green (Fig. 3.E).

3.2. Surface permeabilization

The application of different electroporation protocols to the samples resulted in changes in surface permeabilization. An increasing number of nuclei can be seen in the samples as the electric field treatment intensity increases. An example of this progression is shown in Fig. 4, where 650 V/cm and a pulse width of 50 µs was applied at differing numbers of pulses: 25, 100, 150, and 200. Without the application of electrical treatment, no electroporated nuclei were observed on the leaf surface (Fig. 4.A). The application of electrical protocols with 25 pulses provoked the electroporation of some epidermal cells on the leaf surface (Fig. 4.B) while no guard cell electroporation was observed. The electroporated cells occurred randomly on the surface of the leaves. Increasing the number of pulses to 100 provoked homogeneous permeabilization of the epidermal cells on the leaf surface (Fig. 4.C), while no guard cells were found to be electroporated. When the number of pulses increased further to 150 pulses, the electroporation of some guard cells was observed (circles in Fig. 4.D). When increasing the number of pulses to 200 pulses, the guard cells in the leaf surface were

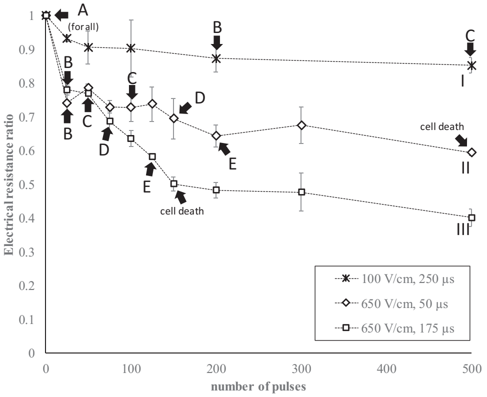

Fig. 5. Changes in resistance with increased number of pulses of PEF treated Thai basil leaves with differing combinations of parameters: I) 100 V/cm and 250 μs pulse width, 760 μs pulse space, II) 650 V/cm and 50 μs pulse width and 760 μs pulse space, and III) 650 V/cm and 175 μs pulse width and 760 μs pulse space. Letters (A-E) represent the different electroporation levels of the leaf surface described in Fig. 4.

homogeneously electroporated (circles in Fig. 4.E), however, not all the tested reversible PEF protocols achieved guard cell electroporation. The number of pulses in which these levels of surface permeabilization occurred were strongly dependent on the combination of voltage, pulse width, and pulse space applied to the samples.

3.3. Application of PEF - Influence of voltage, pulse space and pulse width on the permeabilization of the leaf surface

Typical changes in the electrical resistance ratio of the samples treated with electroporation protocols and the increasing number of pulses are shown in Fig. 5. Three different cases are illustrated, the first (curve I in Fig. 5) shows that, when applying low voltage to the tissues (100 V/cm, 250 μs pulse width, 760 μs pulse space), a slight decrease of the electrical resistance ratio was observed with an increasing number of pulses. Homogeneous epidermal cell electroporation (point C in curve I) was observed in the slowly decreasing part of the resistance curve. Guard cell electroporation (pictures D and E in Fig. 4) was not observed. These results are representative of the electroporation achieved when a voltage of < 300 V/cm and pulse width of < 150 μs were applied. The second curve (curve II in Fig. 5) illustrates the case where leaves were treated with a stronger PEF protocol (650 V/cm, 50 μs pulse width, and 760 μs pulse space with increased number of pulses). A rapid decrease of the R_{ratio} was observed when applying 25 pulses followed by a slower decrease of the R_{ratio} with an increased number of pulses. All surface permeabilization levels described in Fig. 4. (A-E) could be observed. Homogeneous epidermal cell permeabilization was observed in the slowly decreasing part of the R_{ratio} curve (point C in curve II, Fig. 5). These results are representative of the electroporation achieved when applying voltage in the range of 300–650 V/cm, pulse width in the range of 50–150 μs, and 760 μs pulse space at a differing number of pulses. However, guard cell electroporation was observed only in the tissue treated with a specific combination of parameters:650 V/cm, 760 μs pulse space, 50 μs pulse width, and between 200 and 300 pulses. The third curve (curve III in Fig. 5) shows the case where an increased pulse width of 650 V/cm was applied (650 V/cm, 175 μs pulse width, 760 μs pulse space). The R_{ratio} of the tissues decreased rapidly with the increasing number of pulses in the range of 25–150 pulses. All of the surface permeabilization levels in

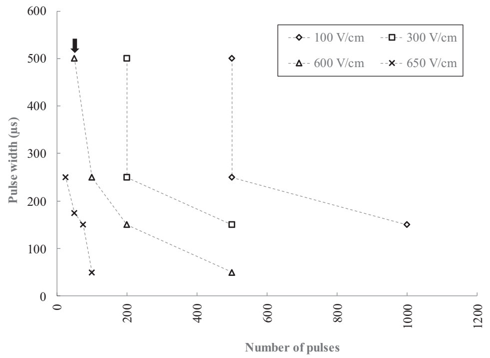

Fig. 6. Number of pulses required to provoke homogeneous epidermal cell permeabilization in Thai basil leaves treated with PEF protocols with differing voltages and pulse widths. The black arrow represents a sample where irreversible electroporation was provoked.

Fig. 4 were observed. Homogeneous epidermal cell electroporation and guard cell electroporation were observed in the rapidly decreasing part of the curve. These results are representative of the electroporation achieved when applying voltage in the range of 600–650 V/cm, pulse width in the range of 150–1000 μs, and pulse space of 760 μs. For every combination of voltage, pulse width, and pulse space, homogeneous epidermal cell permeabilization was observed at a different number of pulses depending on the levels of other parameters. However, only a specific combination provoked guard cell electroporation: 650 V/cm, 175 μs pulse width, 760 μs pulse space, and 100–125 pulses.

The dependence of homogeneous surface permeabilization (of epidermal and guard cells) on the combination of voltage, pulse width, and pulse space is illustrated in Figs. 6 and 7. Each point in Fig. 6 represents a combination of the pulse quantity and pulse width at different voltages needed for the homogeneous electroporation of epidermal cells (Point C in Fig. 5). The arrow in the figure represents the combination of parameters that provoked the death of the cells. For all voltages tested, PEF protocols with longer pulse width required a smaller number of pulses to provoke the homogeneous electroporation of the epidermal cells. An increased number of pulses required shorter pulses.

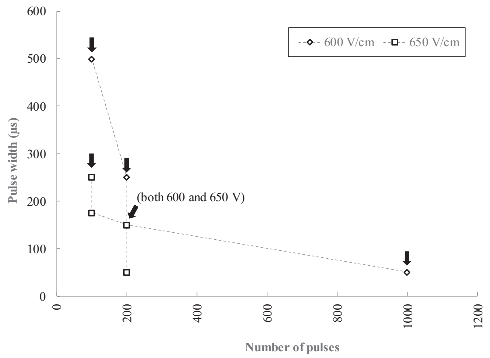

Fig. 7. Number of pulses required to provoke homogeneous guard cell electroporation in Thai basil leaves treated with PEF protocols with differing voltages and pulse widths. Black arrows represent samples with irreversible electroporation.

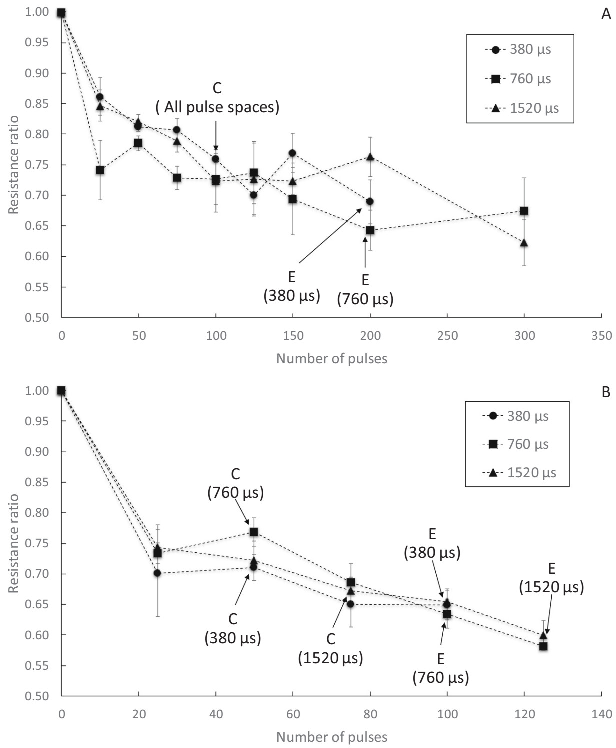

Fig. 8. Changes in tissue resistance in samples treated with PEF protocols at 650 V/cm with a pulse width of 50 µs (A) and 175 µs (B), different pulse spacing (380, 760, and 1520 µs), and an increasing number of pulses were tested. Letters (C, E) represent the different surface electroporation levels shown in Fig. 4.

Depending on the treatment, homogeneous epidermal cell permeabilization was observed in the samples with a R_{ratio} in the range of 0.5–0.8. Each point in Fig. 7 represents a combination of pulse quantity and pulse width at different voltages needed for the homogeneous electroporation of the leaf guard cells (Point E in Fig. 5). The arrows in the figure represent the combination of parameters that provoked the death of the cells. Only two protocols: 1) 650 V/cm, 50 µs pulse width, 760 µs pulse space, 300 pulses and 2) 650 V/cm, 175 µs pulse width, 760 µs pulse space, 125 pulses provoked guard cells electroporation while preserving cell viability and were selected for the drying experiment (PEF1 and PEF2 in Table 2).

3.4. Application of PEF - Effect of space between pulses and number of pulses

Choosing the voltage (650 V/cm) and the two levels of pulse width that were established to provoke the homogeneous permeabilization of guard cells (Fig. 7), the change of the pulse space (380, 760 and 1520 µs) and the number of pulses (0 to 300) was tested. For the protocols with a pulse width of 50 µs (Fig. 8.A), homogeneous epidermal cell permeabilization was observed with the same number of pulses for all pulse spaces tested (point C in Fig. 8.A). Homogeneous guard cell electroporation was found when applying 200 pulses and a pulse space of 380 and 760 µs (point E in Fig. 8.A). No guard cell electroporation was found with a pulse space of 1520 µs. For the protocol with a pulse width of 175 µs (Fig. 8.B), homogeneous epidermal cell permeabilization was observed at the same number of pulses (50 pulses) when applying a pulse space of 380 and 760 µs, while the protocol with a pulse space of 1520 µs provoked homogeneous epidermal cell permeabilization when 75 pulses were applied. Homogeneous guard cell electroporation was observed on the tissues when 100 pulses were applied with a pulse space of 380 and 760 µs (point E in Fig. 8.B), while the protocol with a pulse space of 1520 µs provoked guard cell electroporation when 125 pulses were applied.

3.5. Effect of pulse polarity and number of pulses

The change in tissue resistance with an increasing number of pulses at 650 V/cm, 50 and 175 µs of pulse width, and 760 µs of pulse space in samples treated with monopolar and bipolar protocols are shown in Fig. 9. Samples treated with monopolar pulses showed a higher degree of electroporation than samples treated with bipolar pulses. For the pulse width of 50 µs (Fig. 9.A), homogeneous epidermal cell permeabilization was observed in the samples treated with 100 monopolar pulses compared to 500 pulses for the bipolar protocol. Homogeneous guard cell electroporation occurred when applying 200 monopolar pulses (point E in Fig. 9.A). For the pulse width of 175 µs (Fig. 9.B), homogeneous epidermal cell permeabilization was provoked when 75 and 200 pulses were applied for the monopolar and bipolar protocols respectively. Homogeneous guard cell electroporation occurred when

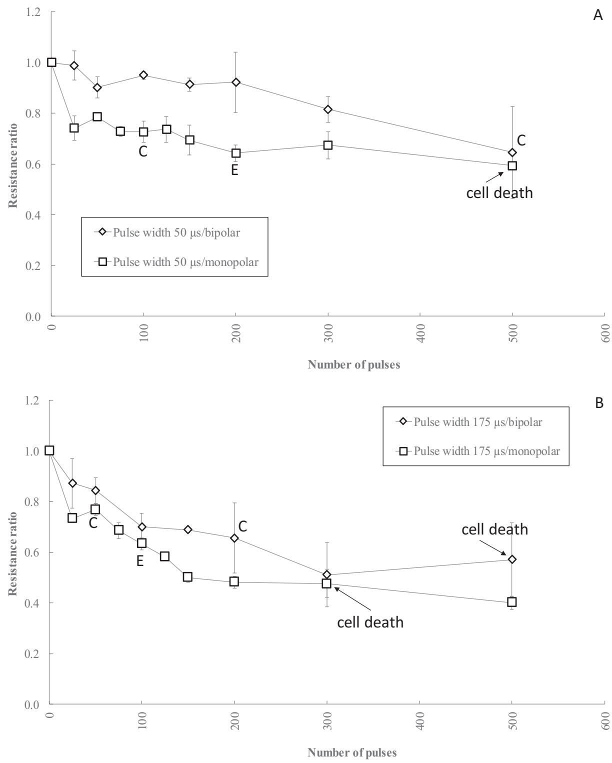

Fig. 9. Changes on tissue resistance in Thai basil samples treated with guard cell electroporation protocols and different pulse polarity. Samples were treated with PEF protocols at 650 V/cm, a pulse width of 50 (A) and 175 (B) µs, a pulse space of 760 µs, and an increasing number of pulses. Letters (C, E) represent the different surface electroporation levels shown in Fig. 4. "cell death" represents the samples in which irreversible electroporation was observed.

applying 100 monopolar pulses (point E in Fig. 9.B). In addition to the 760 µs pulse spacing, 380 and 1520 µs were tested (not shown). Changing the pulse space did not affect the number of pulses required to permeabilize the epidermal cells. The different pulse space tested with the bipolar pulses showed a similar trend in electrical resistance change.

3.6. Application of MEF - Effect of voltage, frequency, and treatment time

The effect of frequency (60, 600, and 1200 Hz), voltage (50–600 V/cm), and treatment time in the MEF treatment on the electrical resistance, cell permeabilization, and viability of the samples was investigated. The R_{ratio} of the MEF-treated samples with increasing treatment times at 1200 Hz is shown in Fig. 10. For all treatment times tested, increasing the voltage applied to the samples decreased the R_{ratio} of the samples. For all voltages and treatment times, changes in frequency showed no effect on the R_{ratio} and the electroporation of the cells at the surface (not shown). No electroporation in the leaf surface was detected when applying 50 V/cm and reversible, homogeneous permeabilization of the leaf surface (Point C in the curve) was only detected when applying 100 V/cm. Electroporation of guard cells was, however, not detected. Cell death occurred at rather low treatment times when applying 300 and 600 V/cm. The protocol with 100 V/cm, 1200 Hz, and 1200 ms of treatment time was selected for the drying experiment (Table 2).

Each point in Fig. 11 represents the treatment time required to provoke homogeneous permeabilization of epidermal cells at different voltages (only 1200 Hz is shown) and longer treatment times. The protocols with higher voltage required shorter treatment time to provoke homogenous epidermal cell electroporation of the leaf surface. However, voltages above 200 V/cm provoked the loss of tissue viability. No guard cell electroporation was found in any of the protocols tested.

3.7. Effect of leaf electroporation on drying

To examine the effect of guard cell electroporation on the drying of Thai basil leaves, the electroporation protocols listed in Table 2 were applied to the samples as a pre-drying treatment. The average moisture content of fresh Thai basil leaves was 91 ± 0.74% (w.b.). The effective moisture diffusivity (D_{eff}), the drying time required to dry the samples to MR of 0.05 (water content of 0.2 kg water/kg dried weight), the average water activity and the final moisture content of the samples are reported in Table 3. The moisture diffusivity of all tested PEF protocols was significantly different ($p < .05$) from each other and from the control; the diffusivity of the MEF protocol did not significantly differ from the control. Electroporation of the guard cells decreased the drying time in comparison with the control: 51% for protocol PEF1 and

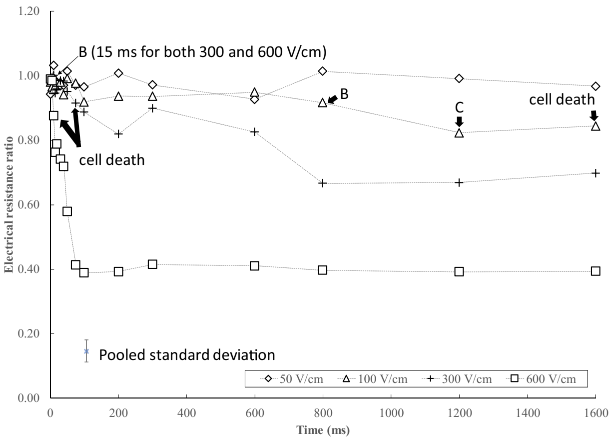

Fig. 10. Changes in resistance with increasing treatment time in samples treated with MEF protocols at different voltages. Letters (B and C) represent the different electroporation levels in the leaf surface described in Fig. 4.

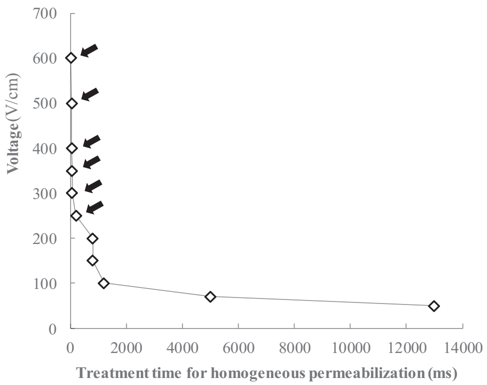

Fig. 11. Treatment time required to provoke homogeneous electroporation of the leaf surface at different voltages for samples treated at a frequency of 1200 Hz. Arrows represent the samples where cell death was detected.

43% for protocol PEF2.

Table 4 reports the values for the sum of the square errors (SSE), root mean square error (RMSE), and R-square of the fitted model. The Page model showed a good fit to the data with the R-square in the range of 0.935–0.999. Drying curves of all protocols are shown in Fig. 12.

Table 4
Statistical parameters and estimated model's parameters calculated from the computed model.

Treatment	n	k (min-1)	SSE	R2	RMSE
Control	0.577	0.00755	3.247	0.998	0.007
PEF1	0.521	0.01972	23.700	0.973	0.025
PEF2	0.471	0.02945	51.286	0.935	0.036
PEF3	0.549	0.00939	22.004	0.989	0.019
MEF	0.499	0.01606	2.943	0.998	0.007

4. Discussion

Our results show that permeabilization of cells on the surface of Thai basil leaves is not an “all-or-nothing” effect. Rather, it is progressive, and its extent depends on the intensity of the electric field and the combination of different parameters, such as pulse width, pulse space, and number of pulses. The cell's response to electric pulses is influenced by cell size, morphology, and orientation (Ben Ammar, Lanoiselle, Lebovka, Van Hecke, & Vorobiev, 2011; Vorobiev & Lebovka, 2008). In the leaf surface, epidermal cells at different development stages can be found in the same leaf (Glover, 2000), and stomata can be found in different sizes, amount, and stages of opening (Weyers & Lawson, 1997).

We have shown that after the progressive permeabilization of the epidermal cells takes place, guard cell permeabilization occurred at a higher number of pulses. Structural features of the leaf epidermis may provide the guard cells with higher resistance to electroporation. Among these features, cuticular folding has been found to form surrounding or overlapping on the top of guard cells and the stomatal

Table 3
Effective moisture diffusivity of the samples from selected protocols.

Treatment	Deff	Drying time (min)	Final moisture content (%)	Water activity (aw)
Control	$2.935 \times 10^{-12} \pm 4.839 \times 10^{-13d}$	544 ± 139^{c}	6.1 ± 0.4^{a}	0.49 ± 0.14
PEF1	$3.485 \times 10^{-10} \pm 1.727 \times 10^{-11a}$	256 ± 9^{a}	7.9 ± 2.0^{a}	0.47 ± 0.05
PEF2	$4.296 \times 10^{-12} \pm 4.469 \times 10^{-13b}$	309 ± 35^{b}	7.7 ± 1.8^{a}	0.48 ± 0.11
PEF3	$2.548 \times 10^{-12} \pm 5.687 \times 10^{-13c}$	510 ± 106^{c}	6.6 ± 1.5^{a}	0.46 ± 0.16
MEF	$3.170 \times 10^{-12} \pm 4.842 \times 10^{-13d}$	504 ± 91^{c}	5.1 ± 2.1^{a}	0.46 ± 0.15

Different letter superscript (a–d) indicates statistical significance ($p < 0.05$).

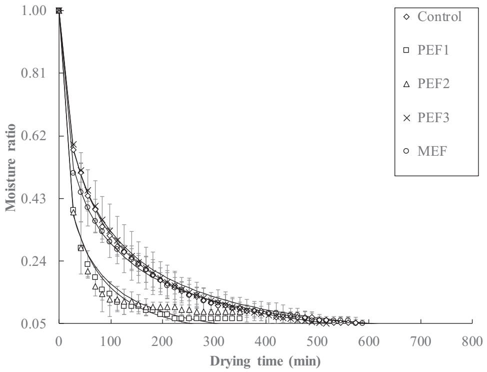

Fig. 12. Drying curve of samples treated with different PEF and MEF protocols described in Table 2. Experimental data are represented with marks while the lines represent the theoretical data calculated on the basis of the model. Reported are average curves for three drying procedures. The variability of the drying data for each condition was < 4%.

complex (Pautov et al., 2019; Wilkinson, 1979). Another aspect that differentiates guard cells from other plant cells is a thicker cell wall (Taiz et al., 2015).

Our results clearly show that the combination of different electroporation parameters is of key importance for the homogeneous permeabilization of the heterogeneous leaf tissue. As the decrease in electrical resistance is dominated by the electroporation of cells in bulk tissues (Dymek et al., 2015), it can be assumed that low voltage PEF provoked lesser electroporation of the cells in the bulk tissues than the higher voltage PEF. For the low voltage PEF (Fig. 5, line I), homogeneous epidermal cell permeabilization was reached only when no further decrease in tissue resistance could be measured (Point C in Fig. 5, Line I), showing that the maximum electroporation of cells in the bulk tissues was more easily reached in comparison to the surface cells.

With increased voltage (Fig. 5, line II and III), the pulse width has a strong influence on tissue electroporation. The application of a 175 μs pulse width (Fig. 5, Line III) provokes homogeneous permeabilization of the leaf surface rather early in the rapidly decreasing section of the resistance curve, showing that the surface is permeabilized before the permeabilization of the bulk of the tissues is completed. The opposite effect is seen when applying a 50 μs pulse width (Fig. 5, Line II), where surface permeabilization took place after no further decrease in tissue resistance could be detected. This result suggests that the highest applied voltage, in combination with long pulse widths, facilitates the electroporation of the different cells in the heterogeneous leaf tissue. However, increasing the pulse space decreased the efficiency with which the protocols electroporated the guard cells (Fig. 8.A). Longer pulse space might allow the membranes to recover back to their pre-electroporated state (Asavasanti, Ristenpart, Stroeve, & Barrett, 2011).

When comparing the polarity of the pulse, bipolar protocols provoked homogeneous surface permeabilization at a higher number of pulses compared to monopolar protocols (Fig. 9). Several studies reported that the electropermeabilization provoked by monopolar pulses associated with PI staining was triggered from the anode pole of the cells (Dymek et al., 2013; Gabriel & Teissie, 1997). The lack of permeabilized cells observed on the cathode-facing may be provoked by the diffusion of PI. If PI molecules enter the tissue from the anode facing side, it may not be able to reach the other side of the leaf within the pulse duration (Dymek et al., 2013). With longer pulse width or higher field intensity, electroporation may progress to reach the cathode facing pole of the cells (Gabriel & Teissie, 1997). On the other hand, the surface permeabilization of samples treated with bipolar protocols was observed on both sides of the leaves, suggesting that the transport of PI molecules induced by bipolar protocols occurred from both the cathode and anode poles of the cells. This observation is supported by the results reported by Vernier, Sun, and Gundersen (2006) showing that bipolar pulses disturb the phospholipid order of the cell membrane at both electro-facing poles of the cell, in contrast with monopolar pulses in which small fluorescent dyes enter cells only through the anode facing side.

The failure to permeabilize stomata guard cells with bipolar pulses applied with PEF and bipolar sinusoidal electric field applied with MEF, suggests that guard cell permeabilization may be possible only with DC monopolar pulses and/or with the high-voltage, short duration treatment of PEF at very specific combinations of pulse width and pulse spacing. There may be some degree of specificity with regard to the effect that the electric field has on guard cells as specialized tissue structures.

Our results show that guard cell electroporation could be used to enhance the drying process of Thai basil leaves. This enhancement has been previously observed by Kwao et al. (2016) and was attributed to permanent stomatal opening during drying. Stomatal opening may be the result of the loss in turgor pressure in the cells surrounding guard cells due to increased permeability during electroporation (Zvitov, Schwartz, Zamski, & Nussinovitch, 2003).

It has been suggested that electroporation may influence the drying process, even if the stomata are not electroporated, due to the temporary damage caused to the cell membranes (Kwao et al., 2016). In our case, the electroporated tissue without guard cell electroporation provoked by PEF protocols showed shorter drying time and higher D_{eff} compared to untreated samples (Table 3). Interestingly, the epidermal cell electroporation provoked by MEF protocols did not show a similar drying enhancement (Table 3), suggesting that MEF may provoke less poration to the cell membrane compared to PEF. As the electrical resistance of the samples treated with MEF at 100 V/cm decreased only slightly with increasing treatment time (Fig. 10), the electroporation of the cells in the tissues may primarily occur on the leaf surface. Similarly, with PEF protocols provoking homogeneous epidermal cell and guard cell electroporation, the D_{eff} of the samples treated with a shorter pulse width (PEF1) was lower than the samples treated with a longer pulse width (PEF2) (Table 3), suggesting that the longer pulse width may provoke higher leakage of the cell membranes resulting in drying enhancement.

5. Conclusions

Our results provide information on the influence of electroporation protocols on the electroporation of Thai basil leaves. We tested the effect of PEF and MEF parameters on the reversible electroporation of Thai basil leaves and, more specifically, on the electroporation of guard cells. The main findings of this research are as follow:

- With PEF and MEF, the electroporation of the leaf surface started with the epidermal cells, and the amount of electroporated cells increased with increasing treatment time. With the PEF treatment, after the epidermal cells were homogeneously electroporated, guard cell electroporation occurred.
- Electroporation of guard cells occurs within a narrow range of electroporation conditions, which are close to the limit between reversible and irreversible permeabilization. With the highest voltage that was applied for obtaining reversible permeabilization, guard cells electroporation was highly dependent on pulse width, number of pulses and pulse spacing. For guard cells to electroporate, increasing pulse width decreased the need of pulses and the longer the space between pulses, the higher the number of pulses needed. Only monopolar protocols were found to electroporate stomatal guard cells.

- Guard cell electroporation can be applied to enhance the drying process of Thai basil leaves. The samples with electroporated guard cells showed a reduction in drying time in the range of 40–50% compared to untreated samples and MEF-treated samples.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Paper III

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The effect of reversible permeabilization and post-electroporation resting on the survival of Thai basil (*O. Basilicum* cv. thyrsiflora) leaves during drying

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ABSTRACT

Horticultural crops have a low tolerance to dehydration. In this paper, we show that the reversible electroporation (200 monopolar, rectangular pulses of 50 μs pulse duration, 760 μs between pulses and nominal field strength of 650 V/cm) of Thai basil leaves followed by 24 h resting before hot air drying at 40 °C enhanced the survivability of the tissues at certain levels of dehydration (moisture ratio = 0.2 and 0.1). However, this increased survival was rather limited. Through measurements of metabolic heat production during resting, rehydration kinetics, respiration and photosynthesis of the rehydrated leaves, we show that resting after the application of a reversible pulse-electric field (PEF) may allow a phase of hardening that has a protective effect on the cells, thus decreasing damage during the subsequent drying phase. Increased preservation of cell vitality would be associated with a more turgid and fresh-like rehydrated product, as cells would have the capacity to retain the rehydration water.

1. Introduction

Electroporation is a food processing technology that can be applied for a variety of purposes, such as the inactivation of microorganisms [1–3] or improvement of extraction yield [4–6]. Drying enhancement is one of the potential applications of electroporation [7–9]. When irreversible permeabilization is applied as a pre-treatment, the viability of cells in the treated tissues is lost, mass transfer is promoted by cell disruption and intracellular leakage occurs, enhancing the drying rate of the food material [10–12]. By strictly controlling the pulsation parameters, the effects of permeabilization on cell viability may be avoided, allowing treated cells to remain viable after electroporation (reversible electroporation). Even if the cell membrane function is restored with time, reversible electroporation has been shown to significantly shorten drying times in basil by provoking the electroporation of stomatal guard cells [10]. Once they are electroporated, stomata remain open during the drying process. This pretreatment obtained dried basil with better aroma and color than the control. Preserved cell viability in the basil leaves treated with reversible electroporation might play an important role in the improvement of the quality characteristics of the dried product.

Mesophytic plants, to which horticultural crops belong, cannot survive a desiccation beyond 20–50% water content in their vegetative parts [13]. Cellular shrinkage, turgidity loss, shriveling of cell walls would provoke structural and textural collapse of the tissue [14]. Moreover, the protoplasm does not regain its original shape after rehydration and the restoration of metabolic activity lost during dehydration has been regarded in previous literature as impossible [15,16]. Therefore, the possibility of achieving preservation of cell vitality after rehydration is a very interesting opportunity and a challenge for technological development in the field; a more turgid and fresh-like (and consequently, with improved quality) rehydrated product would be obtained, as cells would have the capacity to retain the rehydration water and the extent of collapse could be reduced.

If collapse would be minimized and cell vitality restored, a pretreatment prior drying or drying technology should minimize or prevent cell and tissue damage. To the best of our knowledge, research towards optimization of drying methods focusing on tissue damage prevention is scarce in the literature and mostly oriented towards novel pre-drying and drying methods, temperatures and the use of additives or osmotic dehydration,

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influencing the structure and composition of the fresh raw material [17–19].

After it was demonstrated that the function of the stomatal complex can be affected by the distinct application of certain PEF conditions [10], Thamkaew and Gómez Galindo [19] reported a systematic study where several combinations of reversible PEF parameters were tested to identify specific protocols were the homogeneous permeabilization of the leaf surface and the guard cell electroporation occurs, preserving cell viability prior drying. It was demonstrated that, with the highest applied voltage, guard cells electroporation was highly dependent on pulse width, number of pulses and pulse spacing. Once the appropriate combination of parameters was applied, a > 40% reduction in drying time at 40 °C was obtained compared to untreated samples. However, plant cell death or survival in response to a cellular stress depends on complex molecular interactions of a large number of cellular proteins and metabolites [20], and the outcome of influences like PEF and drying cannot be predicted. Consequently, little is known about the influence of the optimized PEF protocol on cell damage, tissue collapse and cell survival upon dehydration, a key aspect of quality.

The study presented in this paper applies the optimal combination of PEF parameters reported by [19] to Thai basil leaves prior to drying and explores whether the faster drying will preserve the viability of the cells after the dehydration process at 40 °C and a following rehydration. A survival of cells upon dehydration would necessarily imply that the application of PEF would confer a certain level of protection to the product during the dehydration, beyond regular levels of desiccation tolerance. In the present study, tolerance to dehydration to various levels of water content was therefore tested after the application of PEF. The effect of the PEF protocol established in the previous publication of our group [19] on electroporation of Thai basil leaves as well as their effect on drying were, for the sake of scientific correctness and reproducibility, scrutinized in the present study by traditional microscopy and analytical methods prior the exploration of cell viability and functionality upon drying.

Phoon et al. [21] showed that reversible permeabilization combined with the impregnation of a cryoprotectant improved the freezing tolerance of spinach leaves. The role of stress acclimation responses associated with the application of PEF was evident, as the cryoprotection effect was detected only when the leaves were frozen 24 h after the application of the PEF treatment. In the present study, the effect of drying the leaves 24 h after the application of PEF was therefore also assessed.

2. Materials and methods

2.1. Raw material

Potted Thai basil (O. *basilicum* cv. thyrsiflora) was grown at a local grower's greenhouse in a controlled environment for 28 days before being transported to our laboratory. The potted plants were placed under LED growth lamps with a light intensity of 200 μmol m^{-2} s^{-1} for 16 h/day under ambient temperature conditions (21 ± 2 °C) for at least 2 days before experimentation to prevent sugar starvation. On the day of experimentation, the plants received light for at least 2 h to initiate stomatal opening. Thai basil leaves with a size of 2.5 ± 0.2 cm × 3.5 ± 0.3 cm and with a mass of 0.18 ± 0.05 g were harvested and used within 15 min after harvest. Before experimentation, harvested leaves were kept in a closed plastic container with wet tissue to prevent moisture loss. All plants were used within 5 days after arriving from the grower.

2.2. Treatments

2.2.1. Electric treatments

Three Thai basil leaves were placed in an electroporation chamber with a 0.5 cm gap between the electrodes. The chamber was filled with 50 ml of NaCl solution (130 μS/cm), which is sufficient to cover both electrodes and samples. The mass-ratio of solution to sample was 100:1. The chamber was then connected to a pulse generator (ADITUS AB, Lund, Sweden) for PEF treatments. The electroporation protocol reported by Thamkaew and Gómez Galindo [19] was applied: monopolar square pulse, number of pulses (n) of 200, pulse width (τ) of 50 μs, pulse repetition frequency (f) of 1234 Hz and amplitude of electric pulse (U) of 650 V/cm. This PEF protocol was reported to reversibly electroporate epidermal cells in Thai basil leaves and provoke the opening of the guard cells of the stomata. The temperature increases during PEF treatments were<2 °K. Treated samples were washed with distilled water and placed on absorbent paper to remove excess water before further processing. This procedure was repeated 11 times to yield enough treated leaves for the drying tests.

2.2.2. Resting

After the PEF treatment, leaves were kept in an air-sealed container with moist tissue paper for 24 h at ambient temperature (21 ± 2 °C) in the dark. After the resting period, the container was placed under the growing light for 2 h before initiating the drying process. Untreated leaves and PEF-treated, unrested leaves were used as controls. PEF-treated, unrested leaves were taken to the drier within 30 min after the PEF treatment. Table 1 summarizes the performed treatments.

2.2.3. Drying

Treated Thai basil leaves were dried in a convective dryer at 40 °C with a constant air flow speed of 3 m/s. Each drying batch consisted of 32 Thai basil leaves, which were evenly placed on a metal drying tray (23 × 33 cm, wire mesh with 5.0 × 5.0 mm square holes) without overlapping. Another tray was placed on top of the drying tray containing the leaves to keep them in place during the drying period. The gap between the two trays was 1 mm. The sample sieve load was 0.076 kg/m^2. The trays were placed on a scale attached to a recording system (RS232 Monitor, EVM Software), which recorded the weight loss of the samples continuously during the drying period. The drying time was dependent on the treatment and the target moisture ratio (MR) levels. Three drying procedures were performed per each experimental condition.

3. Analysis

3.1. Microscopic investigation on leaf surface electroporation and viability of the tissue

Propidium iodide was used as an electroporation indicator to investigate the electroporation of the cells on the leaf surface. Propidium iodide (Sigma-Aldrich, USA, λ_{ex} = 535 nm, λ_{em} = 617 nm) was used to stain the nucleus of the permeabilized cells as

Table 1
List of experimental treatments.

Treatments	Electroporation	Resting
Control	–	–
Control-rested	–	24 h
PEF	Reversible	–
PEF-rested	Reversible	24 h

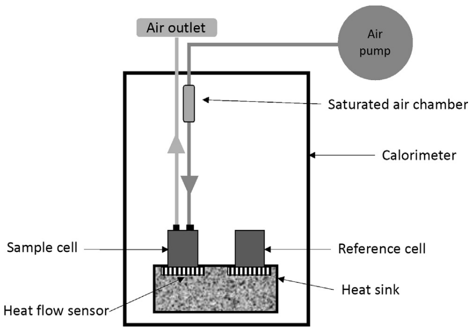
described by Dymek et al. [12]. Three Thai basil leaves were electroporated in a 250 μM PI solution in a 43 μM phosphate PBS buffer with a conductivity (C) of 130 μS/cm. Treated leaves were rinsed with running tap water, and the excess moisture was removed gently with tissue paper. Microscopic observations were conducted using a fluorescence microscope (Elipse Ti-U, Nikon, Japan) at 10 × magnification. The images of the samples were taken with a digital camera (digital sight DS-Qi1Mc, Nikon Co., Japan). All microscopic observations were performed on the bottom-side of the leaves to obtain clear observation of the stomata.

The viability of cells after electroporation was investigated using fluorescein diacetate (FDA; Sigma-Aldrich, USA, λ_{ex} = 492 nm, λ_{em} = 517 nm) as described by Dymek et al. [12]. The prepared FDA stock solution (12 μM) in acetone was stored in the dark at 4 °C. The stock solution was diluted with deionized water to a final concentration of 1.2 nM before the experiment. Leaf samples were electroporated in NaCl solution with a conductivity of 130 μS/cm and then incubated in a closed container with wet paper towels at 4 °C for 20 h. Incubated samples were submerged in the diluted FDA solution in the dark at room temperature for 30 min. The samples were then rinsed with deionized water and examined under fluorescent microscopy. The survival of samples was determined by the occurrence of stained living cells on the leaf surface.

In samples where cell viability was evaluated after dehydration and rehydration, at least 10 micrographs were taken from different locations on each leaf surface with no overlapping of the investigated areas. FDA micrographs were manipulated using ImageJ (V 1.35 a, National Institute of Health, USA). Micrographs were converted into 8-bit images, and automatic image thresholding (Otsu method) was applied with the option "white object on black background" activated. The brightness and contrast of the manipulated images were then adjusted further for improved visualization of the results. For comparison material, cells in the leaves were irreversibly damaged by freezing and thawing.

3.2. Rate of heat production during resting

An isothermal calorimeter (BioCal 2000, Calmetrix Inc., USA) equipped with an air circulation system was used (Scheme 1). Thai basil leaf samples (32 leaves per measurement, 5.17 ± 0.12 g) were placed in a plastic ampule with a volume of 140 ml. The lid of the ampule was connected to an air circulation system, which continuously pumped a constant air flow of 5 ml/min to ensure sufficient O_2 supply to the samples in the ampoules during the measurement. The incoming air passed through a chamber with a wet cloth (66.7% Cellulose, 33.3% cotton), placed inside the thermostat of the calorimeter, to saturate the air flow and prevent moisture loss from the leaf samples. The ampoule with the sample was placed in the measurement chamber at 20 °C, which was connected to the heat flow sensor.



Scheme 1. The calorimeter measurement setup.

The output voltage from the heat flow sensor of the calorimeter was recorded with a computer every minute for 24 h. The thermal power was calculated by Eq. (1)

$$P = \frac{\varepsilon(U_s - U_{bl})}{m} \quad (1)$$

where P is the specific thermal power of the samples (μW g^{-1}), ε is the calibration coefficient of the calorimeter (μW/μV), U_s is the output voltage of the samples (μV), U_{bl} is the output voltage of the baseline (μV), and m is the weight of the samples in the ampoule (g).

The baseline (bl) was recorded before or after each measurement, replacing the sample with 4.8 g of water. The measurements were performed in triplicate.

3.3. Moisture ratio (MR)

The moisture ratio (MR) of the samples during drying was calculated using the Page model [22], assuming that the equilibrium moisture content is negligible. The MR can be calculated as follows:

$$\mathrm{MR} = \frac{M_t - M_e}{M_0 - M_e} = \frac{M_t}{M_0} = \exp(-kt^n) \quad (2)$$

where MR is the dimensionless moisture ratio, M_t is the moisture content at any time (kg water/kg dry mass), M_e is the equilibrium moisture content (kg water/kg dry mass), M_0 is the initial moisture content (kg water/kg dry mass), k is the drying rate constant (min^{-1}), t is the drying time (min), and n is the empirical constant.

The effective moisture diffusivity (D_{eff}, m^2 s^{-1}) was calculated using the simplified Fick's law solution[23] as follows:

$$\mathrm{MR} = \frac{M_t}{M_0} = \frac{8}{\pi^2}\exp(-\frac{\pi^2 D_{eff}}{4L^2}t) \quad (3)$$

where L is the half thickness of the leaves (m) and t is the drying time (s).

Eq. (3) is the solution to the Fick transport equation for an infinite slab. Assuming that the initial moisture distribution is uniform throughout the material, mass transfer is symmetric with respect to the center, constant temperature and diffusivity coefficients, and negligible shrinkage and external resistance [23,24].

The slope (k_0) was calculated by plotting ln MR versus t according to Eq. (4)

$$k_0 = \frac{\pi^2 D_{eff}}{4L^2} \quad (4)$$

The effective moisture diffusivity was calculated by solving Eq.4 within a constant drying rate period (slope of linear part of ln MR vs t curve).

3.4. Moisture content and water activity of the dried samples

To measure the moisture content, hot-air oven method was used. The samples were placed in a hot air convection oven (AB Termo-Glas, Gothenburg, Sweden) at 105 °C for 24 h [25].

The water activity of the leaf samples was measured with an Aqualab (Model CX-2, Decagon devices Inc., Pullman, WA) water activity analyzer at 20 °C. The analysis was done in triplicate.

3.5. Rehydration capacity

The rehydration capacity of dried samples was determined using the method described by Telfser and Gómez Galindo

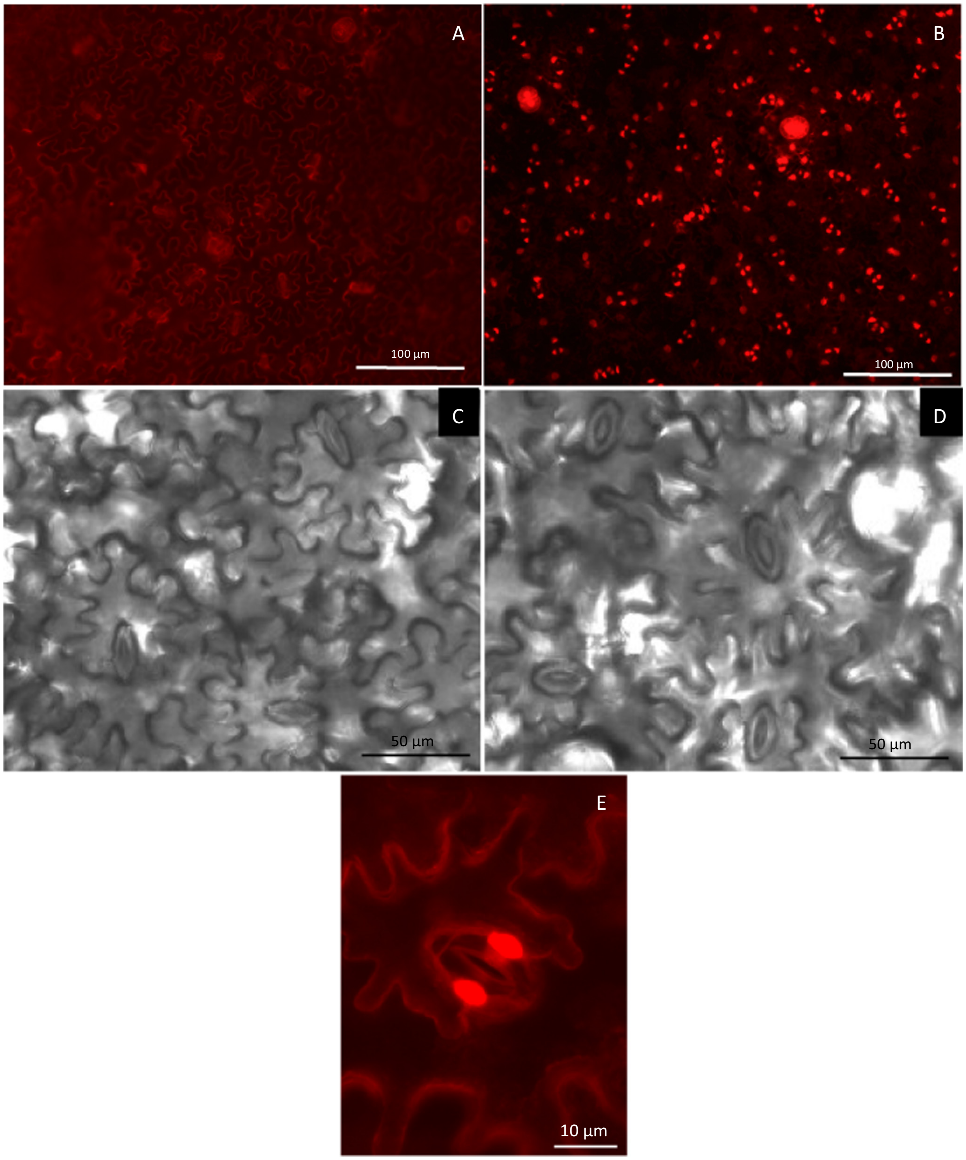


Fig. 1. Representative micrographs from PI staining showing electroporation and bright-field images showing open or closed stomata on the surface of Thai basil leaves. (**A**) Untreated control with no electroporation. (**B**) Leaf samples with homogeneous epidermal and guard cell electroporation. (**C**) Bright-field micrograph of control (untreated). Thai basil leaf showing stomatal closure after the resting period. (**D**) Electroporated Thai basil leaf showing open stomata after the resting period. (**E**) Electroporated guard cells of a stoma appear as two red dots adjacent to each other.

(2019) [26] with some modifications. Each individual dried Thai basil leaf was weighed and placed in a 50 ml plastic tube (2.9 cm in diameter and 11.4 cm in length) filled with 19 ml distilled water at room temperature. The water to leaf ratio was 100:1 by mass. Every hour, leaf samples were taken out from each tube, and the excess water on the leaf surface was removed with tissue paper.

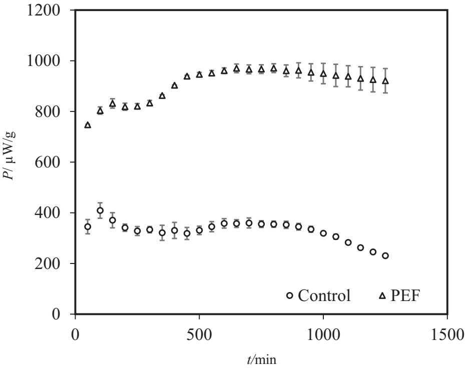


Fig. 2. Specific thermal power (*P*) of Thai basil leaf samples treated with PEF (open triangle) and control (open circles) during the resting period (24 h). The leaf samples were supplied with constant humid air flow during the measurement. Reported are the average curves of three measurements. Error bars in each data point represent the standard deviation of the mean.

The leaf was weighed and placed back into the same rehydration tube. This measurement was repeated until a constant leaf weight was achieved. The test was performed in triplicate for each treatment reported in Table 1. Rehydration curves were created for each treatment by plotting the moisture content (kg water/ kg dry matter) of rehydrated samples against the rehydration time.

To ensure that every leaf sample was rehydrated to the maximum rehydration capacity, the longest rehydration time among all treatments was used as a rehydration time for further investigations (electrical conductivity and photosynthesis).

3.6. Conductivity

After rehydration was completed, each leaf was taken out from the tube and the electric conductivity of the rehydration water was measured at 21 °C with a conductometer (Orion Research Inc., Jacksonville, FL, USA). The leaf samples were placed back into their rehydration tubes until the photosynthesis and respiration measurements were performed (not longer than 30 min).

3.7. Photosynthesis and respiration

The photosynthesis and respiration of the leaf samples were determined using the method described in Panarese et at. (2014) [27] with some modifications. The leaf samples were kept in darkness for 20 min before the measurement. Measurements were performed at 20 °C using an oxygen electrode (S1 O_2 electrode, Hansatech, Norfolk, UK) equipped with a thermostated electrode chamber (LD2/3, Gas-Phase Oxygen Electrode Chamber) and a built-in light source with a light intensity of 380 µmol m^{-2} s^{-1} (LS3 Computer Controlled UV Light Source, Hansatech, Norfolk, UK). Thai basil leaf samples were cut into leaf discs with a diameter of 3.5 cm and placed on a fabric plate soaked with bicarbonate buffer at pH 9. The buffer was prepared with one part 0.4 M $NaBO_3$-buffer (pH 9) and two parts 1 M Na_2CO_3-buffer (pH 9). The measurement started with the dark respiration measurement for 10 mins (light off), followed by the photosynthesis measurement for 10 mins (light on). The results were expressed as oxygen generation (for photosynthesis) and oxygen consumption (for respiration) (nmol(O_2) min^{-1} cm^{-2}). The O_2 electrodes were calibrated before measurement using air and N_2. For each experimental condition listed in Table 1 and each MR level (MR of 0.1 and 0.2), three drying procedures were performed (for a total of 24 drying procedures). For each drying procedure, seven leaves were randomly selected and after rehydration, measured for their photosynthesis and respiration rates individually.

3.8. Statistical analysis

Statistical significance (One-way ANOVA) was performed using SPSS (v.25.0, IBM Corp., Armonk NY, USA) at a significance level of 0.05. Post-Hoc tests were performed using the Tukey-HSD method. Curve fitting was performed using the MATLAB curve fitting toolbox (MATLAB R2019a, MathWorks Inc., MA, USA).

4. Results

4.1. Microscopic investigation

Cells that have taken up PI in their nuclei after electroporation are assumed to have been at least transiently permeabilized in response to the electric pulses. Fig. 1**A** shows the untreated control with no electroporation and no PI staining. The nuclei of permeabilized cells appear as red dots on the micrographs of electroporated tissue (**1B and 1E**). Under the applied PEF protocol, there was uniform electroporation of guard cells, visible as pairs of red dots with approximately 10 µm distance between them (Fig. 1**B**). After the resting period, the bright-field micrographs of control samples showed that most of the stomata were closed (Fig. 1**C**), while the stomata opening effect of reversible PEF persists after resting (Fig. 1**D**). Fig. 1E focuses on one stoma, showing electroporated guard cells as two red dots adjacent to each other in the guard cells.

4.2. Metabolic heat production during resting

The rate of metabolic heat production of PEF-treated and control samples during the resting period (24 h) before drying is shown in Fig. 2**.** The specific thermal power of the PEF-treated leaves is nearly three times the thermal power of the control sample throughout the resting time.

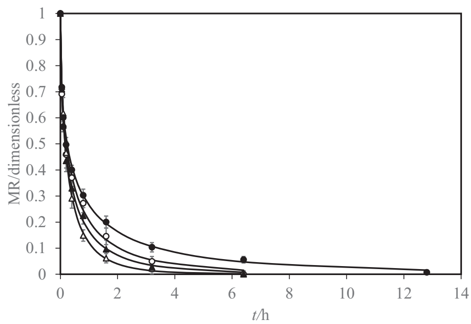


Fig. 3. Drying curves of control (open circles), PEF (open triangle), control-rested (closed circles), and PEF-rested (closed triangles) Thai basil leaf samples as listed in Table 1. Experimental data are represented with marks while the lines represent the theoretical data calculated on the basis of the model. Reported are average curves and standard deviation of the mean from three replications.

Table 2
Statistical parameters and estimated model parameters for drying, calculated from the computed model.

Treatments	k (min^{-1})	n	SSE	R^2	RMSE
Control	0.022	0.523	6.957	0.986	0.0155
PEF	0.014	0.613	1.660	0.996	0.0076
Control-rested	0.034	0.449	2.624	0.996	0.0066
PEF-rested	0.021	0.541	2.513	0.995	0.0091

Table 3
Effective moisture diffusivity, water activity and predicted drying time required to achieve different target MR (0.1 and 0.2) obtained for different treatments. The results were calculated based on Page's model using the fitted parameters shown in Table 2.

Treatments	Drying time (min) MR = 0.2	a_w	Drying time (min) MR = 0.1	a_w	Moisture diffusivity (D_{eff}, m^2 s^{-1}) $\times$ 10^{-12}
Control	62 ± 13[b]	0.84 ± 0.034	123 ± 22[b]	0.61 ± 0.013	4.1 ± 0.6[ab]
PEF	38 ± 6[a]	0.83 ± 0.006	69 ± 16[a]	0.60 ± 0.001	7.2 ± 2.4[b]
Control-rested	92 ± 14[c]	0.86 ± 0.017	205 ± 18[c]	0.60 ± 0.013	2.6 ± 0.2[a]
PEF-rested	49 ± 12[a]	0.82 ± 0.034	95 ± 25[ab]	0.61 ± 0.016	5.4 ± 1.1[ab]

*Different letter superscript within a column indicates statistically significant differences ($p < 0.05$).

4.3. Effect of leaf electroporation and resting on drying.

The treatments listed in Table 1 were applied to the samples as a pre-drying treatment. Drying characteristic curves are shown in Fig. 3. The computed parameters from the data fitted to Page's model [22] for each treatment are shown in Table 2.

Electroporated samples (PEF and PEF-rested) showed significantly shorter drying times compared to non-electroporated samples (control and control-rested). Control-rested and PEF-rested samples showed lower moisture diffusivity compared to unrested samples (control and PEF-treated). The moisture diffusivity of each treatment and predicted drying time to achieve target MR levels are shown in Table 3. These drying times were used in the subsequent experiments.

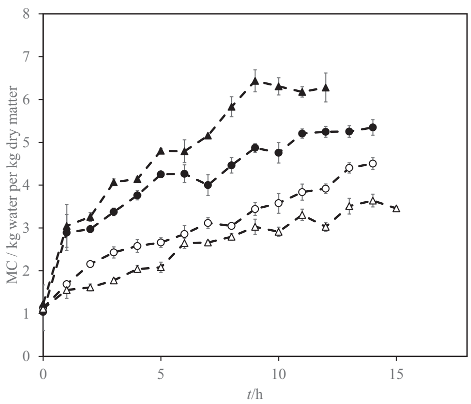


Fig. 4. Rehydration curves of control (open circles), PEF (open triangles), control-rested (closed circles), and PEF-rested (closed triangles) Thai basil leaf samples as listed in Table 1 and dried to MR = 0.1. Reported are average curves for three drying procedures. Error bars in each data point represent the standard deviation of the mean.

4.4. Rehydration capacity.

The rehydration curves of the leaf samples dried to an MR of 0.1 are shown in Fig. 4. Resting significantly increased ($p < 0.05$) the rehydration capacity of both PEF and non-PEF dried leaf samples. The PEF-rested leaf samples showed the fastest rehydration, which reached its maximum at 9 h, followed by the control and control-rested samples at 14 h and PEF-treated samples at 15 h. The maximum rehydration capacity of samples for all treatments is shown in Table 4. For an MR of both 0.1 and 0.2, PEF-rested samples showed the highest rehydration capacity, followed by controlled-rested and control. PEF samples showed the lowest rehydration capacity.

4.5. Conductivity

The electrical conductivity (C) of the rehydration water from leaf samples measures the release of ions from the tissue and is shown for all treatments to MR levels of 0.1 and 0.2 in Table 5. There are no statistically significant differences between the conductivity of rehydration water from the control and the control-rested samples for either MR level. Rehydration water from PEF-rested samples showed significantly lower conductivity for both MR levels of 0.1 and 0.2. Comparing the conductivity of PEF and PEF-rested samples, the resting process decreased the conductivity outcome by 50% and 30% for the samples that were dried to an MR of 0.2 and 0.1, respectively. The conductivity value for the PEF-treated sample is the closest to the leaves that were frozen and thawed, although significantly lower.

Table 4
Maximum rehydration capacity of Thai basil samples treated as listed in Table 1 and dried to MR of 0.1 and 0.2.

Treatments	Maximum rehydration capacity(kg water/ kg dry matter) *	
	MR = 0.2	MR = 0.1
Control	8.05 ± 0.33[a]	4.50 ± 0.20[a]
Control-rested	9.17 ± 1.99[a]	5.35 ± 0.05[b]
PEF	4.22 ± 0.17[b]	3.64 ± 0.23[c]
PEF-rested	11.6 ± 0.78[c]	6.28 ± 0.29[d]

* Different letter superscript in each column indicates statistically significant differences ($p < 0.05$).

Table 5
Electrical conductivity of the rehydration solution of Thai basil leaves treated with different treatments listed in Table 1 to an MR of 0.1 and 0.2. Each conductivity value is the average of three replications.

Samples	Conductivitytsave	
Fresh leaves	6 ± 1.1	
Frozen and thawed leaves	135 ± 9.1	
	MR = 0.2	MR = 0.1
Control	74 ± 6.2^{a}	103 ± 11.6^{a}
Control-rested	69 ± 6.9^{a}	97 ± 14.4^{a}
PEF	100 ± 9.3^{b}	120 ± 14.6^{b}
PEF-rested	51 ± 4.8^{c}	86 ± 11.9^{c}

*different letter superscript in each column indicates statistically significant differences ($p < 0.05$).

4.6. Photosynthesis and respiration

Fig. 5 shows the photosynthesis and respiration rates of the rehydrated samples for all the treatments (MR of 0.1 and 0.2). Among all samples at the MR level of 0.1, only the PEF-rested samples were found to have consumed oxygen during the respiration test, while the respiration and photosynthesis of all the other samples were not detectable. The respiration of PEF-rested samples at an MR of 0.1 was approximately 8% of the fresh samples, and no photosynthesis was detected. At the MR level of 0.2, the samples from the PEF-rested treatments showed significantly higher respiration and photosynthesis capability compared to the samples from all other treatments ($p < 0.05$). At this MR, the photosynthesis and respiration of the PEF-rested samples were approximately 25% of the fresh samples. Low levels close to the detection limit of photosynthesis and respiration were detected in PEF-treated samples that were dried to an MR of 0.2. Respiration and photosynthesis of both control and control-rested samples were detected at the MR of 0.2 without significant differences between them.

Fig. 6**A and 6B** show the values of oxygen consumption and oxygen generation as a function of the electrical conductivity of the rehydration water. In Fig. 6**A**, the leaf samples consumed O_2 during the measurement as the respiration occurred. Fig. 6**B** shows the O_2 generation due to photosynthesis. The figure shows a clear correlation between the capacity of cells to take up and retain the rehydrating water, represented by the conductivity data, and the capacity of the cells to respire and photosynthesize.

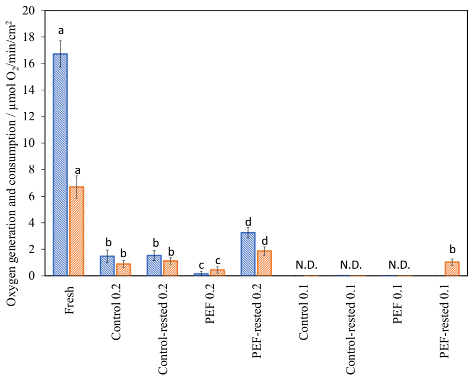


Fig. 5. Oxygen generation of Thai basil leaf samples treated as listed in Table 1, measured with light source on (photosynthesis, blue bars), and oxygen consumption measured with light source off (respiration, red bars). N.D.: not detectable. Reported are average values from 21 measurements. Error bars in each data point represent the standard deviation of the mean. Different letter superscripts indicate statistically significant differences ($p < 0.05$).

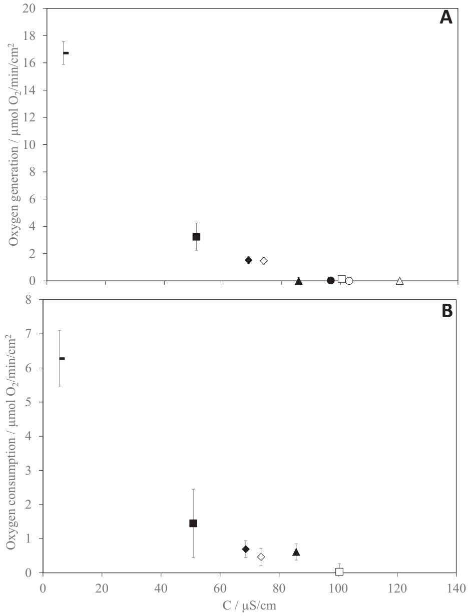


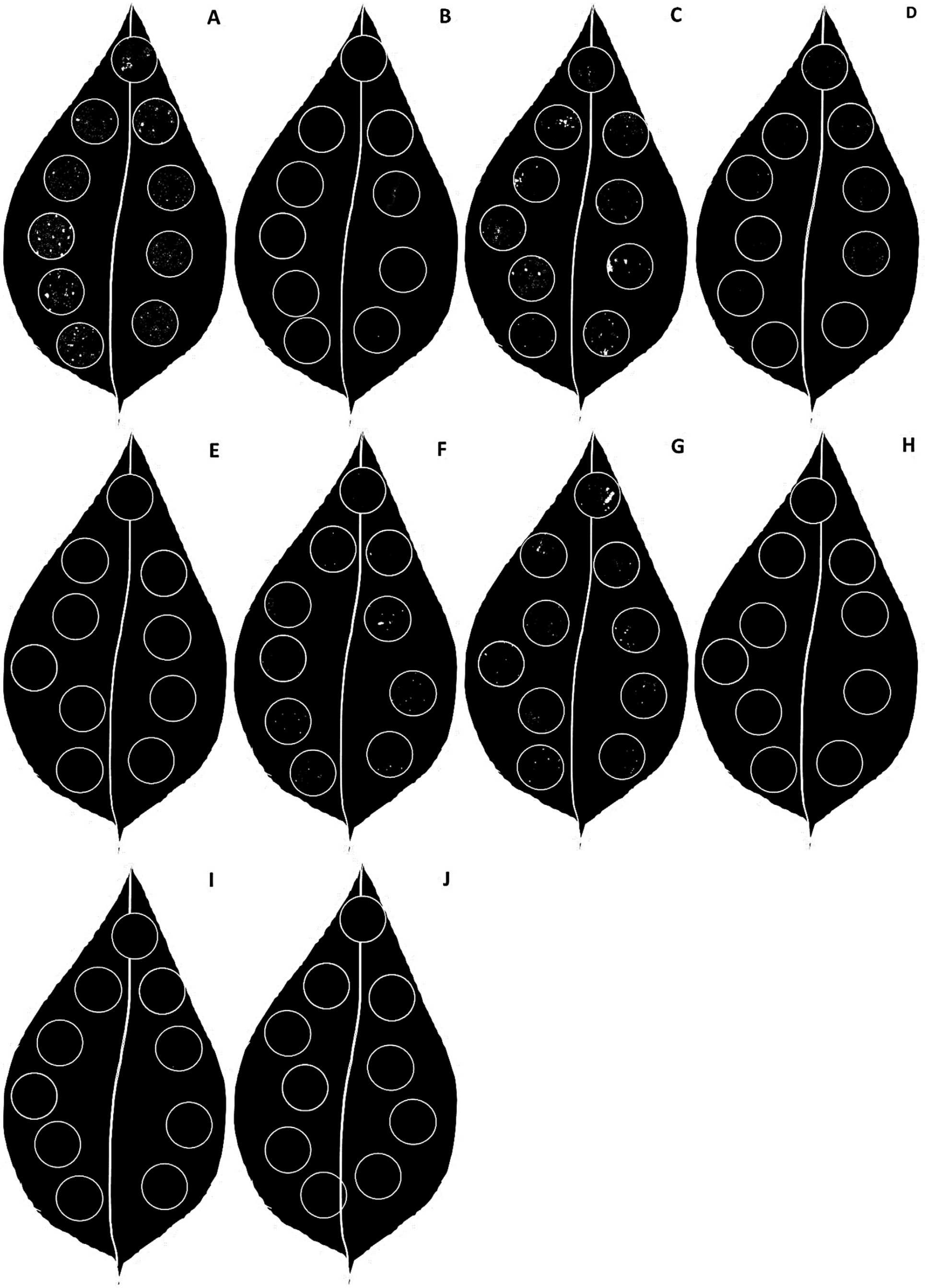
Fig. 6. The photosynthesis (**6A**) and respiration (**6B**) rates of fresh (dash), control and PEF treated Thai basil leaf dried to MR of 0.1: control (open circles), control-rested (closed circles), PEF (open triangles), PEF-rested (closed triangles) and MR of 0.2: control (open rhombuses), control-rested (closed rhombuses), PEF (open squares), PEF-rested (closed squares) as a function of the electrical conductivity (C) of their rehydration water. Reported are average results from three drying procedures. Error bars in each data point represent the standard deviation of the mean.

4.7. Cell viability on the leaf surface

Microscopic observations of cell vitality of the leaf surface for each treatment are shown in Scheme 2. Living cells (white spots) can be seen in all examined fields on the fresh leaf samples (Scheme 2A); compared with leaves that were previously frozen and thawed that show no white spots (Scheme 2B). At the MR of 0.2 (Scheme 2C-2F), more living cells can be found in the PEF-rested samples (Scheme 2C) compared to the other samples. Some living cells were found in the control and control-rested leaf samples (Scheme 2D, 2E) but not in the PEF treated leaf samples (Scheme 2F). At the MR level of 0.1 (Scheme 2G-2J), living cells were found only in PEF-rested samples (Scheme 2G), while all other leaf samples were visually similar to leaves that were previously frozen and thawed.

5. Discussion

It has been well established that the drying time of Thai basil leaves decreased with the application of reversible PEF when the guard cells of stomata are electroporated [10,19]. However, it is only in a very narrow range of electroporation conditions, close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to the limit between reversible and irreversible permeabilization, when opening of stomata occurs [19]. In this paper, we investigate the longer-term physiological outcomes, and show that treating the tissue within these limits may provoke a long-term metabolic response in the cells that provide them with a cross-tolerance seen as an increased protection against dehydration damages.

Comparing the effective moisture diffusivity of PEF-rested samples to all other treatments, it is clear that the drying enhancement effect of PEF remained after the resting period. It should be noted that during resting, all samples showed 12–15% mass gain. This uptake of water vapor from the atmosphere might have contributed to the increased drying time of rested samples in comparison with the non-rested leaves.

Rehydration capacity is one of the most important properties of dried herbs [28–30]. It is well known that the exposure of samples to elevated temperatures provokes damage to cells and the tissue structures, which results in decreased rehydration capacity [31]. However, in our case, the PEF-treated samples that showed the highest drying rate showed the lowest rehydration capacity. This indicates that the combination of electroporation and the elevated temperature of the drying process may have caused the highest damage to the tissue and cells among all treatments. Due to the transient permeabilization of membranes, PEF is expected to cause a temporary drastic loss of metabolic homeostasis, and cells may not have had enough time to recover from the electric treatment when dehydration was started immediately afterwards. Remarkably, the PEF-rested sample showed the highest rehydration capacity among the samples from all treatments, suggesting that resting after the application of reversible PEF may allow a phase of hardening, having a protective effect on the cells and decreasing damage during drying. This result is supported by the fact that the lowest conductivity was measured in rehydration water from the PEF-rested (Table 5), as well as the data for respiration and photosynthesis (Fig. 5), which showed that some degree of cell functionality is better preserved when the leaves are allowed to rest between PEF and drying. This functionality is provided by the surviving cells, some of which are observed with the vitality staining of the leaf surface (Scheme 2). Resting affected the conductivity of PEF-treated samples but not the conductivity of the control samples (Table 5), suggesting that, despite the effects of resting on the drying and rehydration characteristics of both PEF and control samples, only PEF-treated samples seem to develop this protective mechanism.

Photosynthetic activity is one of the most temperature sensitive plant cell functions. When damaged, plant leaves may partially or completely lose their photosynthetic capability [32], but damaged tissues will continue to respire as long as they are alive. Our results showed that at the MR of 0.1, respiration was detected only in the PEF-rested samples (Fig. 5); however, their respiration was very low compared to fresh samples (approximately 8%), showing that the drying process caused significant damage to the cells in the tissues. Photosynthesis was not detected in any samples at the MR level of 0.1 but was detected in most samples dried to an MR of 0.2. Interestingly, the PEF-rested protocol was able to preserve approximately 25% of both respiration and photosynthesis compared to fresh leaves. Despite the differences in the drying times and rehydration capacity, the respiration and photosynthesis of the control and control-rested samples at an MR of 0.2 were similar. This result suggests that the cell vitality of the samples without PEF is not affected by the resting process.

It has been shown that, after PEF-mediated permeabilization, various physiological events associated with stress responses take place in the cells long after resealing (for a review on stress responses, see [33]), with evidence pointing towards a cell that, after permeabilization and resealing, becomes cross-tolerant and thus more protected against other abiotic stresses [21] or even against a second PEF application [34]. Using isothermal calorimetry, Gómez Galindo et al. [35] showed that when reversible permeabilization was applied to potato tissue, the metabolic heat rate increased and was kept high throughout the 40 min measurement. Dymek et al. [36] also showed a significant increase in the metabolic heat production of vacuum impregnated spinach upon electroporation, an effect that lasted throughout the 20 min of experimentation. In both studies, this increased metabolic activity was associated with stress responses.

In this study, we have modified the calorimeter setup to allow long-term measurements, providing the samples in the calorimetry ampoules with a constant supply of humid air (Scheme 1). In this way, we show an increased and rather steady metabolic heat production of PEF-treated basil, lasting the 24 h between PEF application and drying. The elevated metabolism indicates an increased mobilization of energy that could have allowed for the synthesis of protective compounds over the 24 h resting period. This result may thus reflect a metabolism reacting to the stress provoked by the PEF application, inducing a stress acclimation and cross-tolerance in cells, thus having a better capacity to survive a following dehydration. Future studies should elucidate these protective mechanisms.

Maintenance of cell integrity and properties would allow keeping the product quality close to that of the original product, contributing to efforts to maintain the nutritional value, the typical aroma characteristics and minimize changes in color and texture. However, under the studied PEF pre-treatment parameters and drying conditions, the reported increased survival was rather limited, and more studies are needed seeking for survival improvement, for example by combining the reversible PEF pre-treatment with other drying techniques known to better preserve the structure of the dried tissue such as supercritical carbon dioxide or microwave-assisted drying (for a review on drying techniques for herbs, see [19].

6. Conclusion

The results presented here show evidence that reversible electroporation of Thai basil leaves followed by 24 h of resting before drying enhanced the survivability of the tissues at certain levels of dehydration. However, this increased survival was rather limited. This study can serve as the basis for further investigations on the defense-related consequences of PEF-induced stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Scheme 2. Representative micrographs of vitality staining on Thai basil leaves: (A) fresh, (B) frozen and thawed, (C) PEF-rested MR 0.2, (D) control-rested MR 0.2, (E) PEF-treated MR 0.2, (F) control MR 0.2, (G) PEF-rested MR 0.1, (H) control-rested MR 0.1, (I) PEF-treated MR 0.1, and (J) control MR 0.1. Each circle represents micrographs of the corresponding location in the leaf samples. The micrographs were manipulated with the algorithm described in the Materials and methods section.

Acknowledgement

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Paper IV

Article

Reversible electroporation and post-electroporation resting of Thai basil leaves prior convective and vacuum drying

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Abstract: Pre-treatment by reversible electroporation followed by resting (storage under saturated moisture at 21 ± 2 ºC) was evaluated for modification of the properties of dried and rehydrated Thai basil leaves. The treated leaves were dried by convection at 40 ºC or in vacuum at room temperature. The results showed that vacuum drying provoked more cell damage and tissue collapse than convective air drying at moisture ratio (MR) of 0.2 and 0.1. Under this level of MR, the pulsed electric field (PEF) and resting pre-treatment exerts a protective effect of the tissue for both drying methods. However, under complete dehydration (water activity, aw = 0.05) damage seems to be similar for both drying methods despite the PEF pre-treatment. Remarkably, reversible electroporation followed by resting resulted in higher trichome preservation, showing that this pre-treatment still exerts a protective effect on trichomes when complete dehydration is achieved.

Keywords: Pulsed electric field, trichomes integrity, drying methods, stress response, Thai basil

1. Introduction

Pulsed electric field (PEF) has been used as pre-treatment to increase the rate of mass transfer during drying of foodstuffs such as vegetables, meat, fruit, and herbs [1-7]. However, the majority of these PEF applications were designed to cause irreversible electroporation of cells, which would greatly increase mass transfer but result in numerous changes in food quality, including aroma, color, and texture[4]. Irreversibly electroporated sweet basil leaves appear to lose their aroma and color significantly when compared to untreated and reversibly electroporated samples upon drying in air at 50 ºC[4].

By inducing electroporation on guard cells of the stomata and, at the same time, keep the rest of the cells viable, reversible electroporation could be used to improve the drying of plant leaves[4]. The electroporated guard cells cause long-term stomatal opening, which reportedly aids in the drying process[8]. Telfser and Gómez Galindo (2019)[8] evaluated the effects of reversible electroporation on the structure, rehydration capacity, color, and sensory quality of basil leaves dried using convective drying at 40 ºC, vacuum drying, and freeze-drying. The authors found that reversible electroporation causing stomatal opening of sweet basil leaves reduced the drying time in all studied drying methods, and PEF resulted in better preservation of the leaf structure when used prior to convective and vacuum drying, as compared to the untreated control. In a previous paper, Thamkaew et al. (2020)[9] reported that reversible electroporation followed by resting (storage under saturated moisture at 21 ± 2ºC) allows survival of cells in Thai basil leaves at certain levels of dehydration (aw > 0.6). Resting after PEF and prior to drying allowed the cells to establish protective mechanisms in response to the temporary loss of metabolic homeostasis caused by the electroporation process. Maintenance of cell integrity and properties would allow keeping the product quality close to that in the original product.

Structural integrity of the epidermal and cuticle layers of herbs may be an important aspect for the preservation of aromatic volatile compounds during drying, mainly regarding the integrity of trichomes that produce and accumulate terpenoid oils [10]. The integrity of the trichomes is strongly influenced by the drying conditions and the drying method in lemon verbena [11]. In basil, vacuum drying resulted in a better preservation of the integrity of trichomes than hot air drying at 40 ºC [8]. Vacuum drying is suitable for heat-sensitive food materials[12], having the advantage of low drying temperatures and time, improving the preservation of color [12].

In this study, the documented advantages of reversible permeabilization and post-electroporation resting [13] on cell preservation are tested for complete dehydration (aw = 0.05) using both air drying at 40 ºC and vacuum drying at room temperature. High-resolution optical microscopy with digital 3D surface reconstruction was used as a non-invasive and non-destructive method for evaluating the integrity of trichomes in the leaves after dehydration and rehydration. Other strategies for trichome evaluation such as chemical fixation, scanning electron microscopy, and transmission electron microscopy require extensive sample preparation processes, which may significantly affect the trichomes. We aim at comparing the properties of the dried and rehydrated products using reversible PEF and resting prior to dehydration.

2. Materials and Methods

2.1. Raw material handling

Before being transported to our laboratory, potted Thai basil (O. basilicum cv. thyrsiflora) was grown in a controlled environment for 28 days at a local grower's greenhouse. To avoid sugar starvation, the potted plants were placed under LED growth lamps with a light intensity of 200 μmol m^{-2} s^{-1} for 16 h per day under ambient temperature conditions (21 ± 2 ºC) for 2–5 days before experimentation. The plants were exposed to light for at least 2 h on the day of the experiment to initiate stomatal opening. Thai basil leaves measuring 2.5 ± 0.2 cm x 3.5 ± 0.3 cm in length and weighing 0.18 ± 0.05 g were harvested and examined within 15 min. To prevent moisture loss, harvested leaves were kept in a closed plastic container with wet tissue prior to experimentation.

2.2. Electrical treatment

Three Thai basil leaves were PEF-treated in an electroporation chamber with a 0.5 cm gap between electrodes. The chamber was filled with 50 ml of NaCl solution (130 μS/cm), enough to cover both the electrodes and the leaves. The solution to sample mass ratio was 100:1. For PEF treatments, the chamber was then connected to a pulse generator (ADITUS AB, Lund, Sweden). The PEF protocol reported by Thamkaew and Gómez Galindo (2020) [9] was used: 200 monopolar, rectangular pulses with a 50 μs pulse duration, 760 μs pulse spacing, and a nominal field strength of 650 V/cm. This PEF protocol was found to reversibly electroporate epidermal cells in Thai basil leaves and cause stomatal opening of guard cells[9]. During PEF treatments, the temperature increased by less than 2 K. Before further processing, the treated samples were washed with distilled water and placed on absorbent paper to remove excess water. To obtain enough treated leaves for the drying tests, the PEF procedure was repeated 11 times.

2.3. Resting

After the PEF treatment, the leaves were kept in the dark for 24 h in an air-sealed container with moist tissue paper at room temperature (21 ± 2 °C) ("PEF-rested" leaves). Untreated leaves ("control-rested") were stored under the same conditions in another container. Fresh leaves ("control") were harvested and dried without resting. The containers were placed under the growing light for 2 h before dehydration.

2.4. Drying

2.4.1. Convective drying

Thai basil leaf samples were dried at 40 °C with a constant air flow speed of 3 m/s in a convective dryer built at Lund University. Each batch of leaves was evenly distributed on a metal drying tray (23 x 33 cm, wire mesh with 5.0 x 5.0 mm square holes) without overlapping. To keep the leaves in place during the drying process, another tray was placed on top of the drying tray containing the leaves. There was a 1 mm gap between the two trays. The sample load was 0.076 kg/m^2. During the drying period, the trays were placed on a scale connected to a recording system (RS232 Monitor, EVM Software), which continuously recorded the weight loss of the samples. The drying time was dependent on the treatment and the final moisture ratio (MR). For each treatment: "PEF-rested", "control-rested" and "control", three drying procedures were carried out for each experimental condition and for each MR level (MR of 0.05, 0.1, and 0.2), for a total of 18 drying procedures.

2.4.2. Vacuum drying

Thai basil leaf samples were dried in a vacuum dryer (model will be added) at ambient temperature (21 ± 2°C). The chamber was set to a vacuum pressure of 13 Pa. The leaf samples were arranged on the metal tray in a manner similar to that of convective drying. The drying times were stablished in preliminary experiments aiming at obtaining samples with water activity and MR similar to the air dried samples. Three drying procedures were carried out for each experimental condition and for each MR level (MR of 0.05, 0.1 and 0.2), for a total of 18 drying procedures.

2.5. Analysis

2.5.1. Moisture ratio

The Page model [14] was used to calculate the MR of the samples during drying, assuming that the equilibrium moisture content is negligible. The MR can be calculated using the following equation:

$$\mathrm{MR} = \frac{M_t - M_e}{M_0 - M_e} = \frac{M_t}{M_0} = \exp(-kt^n) \qquad (1)$$

where MR is the dimensionless moisture ratio, M_t is the moisture content at any time (kg water/kg dry mass), M_e is the equilibrium moisture content (kg water/kg dry mass), M_0 is the initial moisture content (kg water/kg dry mass), k is the drying rate constant (min-1), t is the drying time (min), and n is the empirical constant.

2.5.2. Moisture content and Water activity

Moisture content was determined by placing 2 g of samples at 105 °C for 24 h in a hot air convection oven (AB Termo-Glas, Gothenburg, Sweden). The water activity of the leaf samples (2 g) was measured at 20 °C using an Aqualab water activity analyzer (Model CX-2, Decagon devices Inc., Pullman, WA). Measurements of each experimental condition were repeated three times.

2.5.3. Rehydration

The method described by Telfser and Gómez Galindo (2019) [8], with some modifications, was used to determine the rehydration capacity of the dried samples. Each individual dried leaf was weighed before being placed in a 50 mL plastic tube (2.9 cm in diameter and 11.4 cm in length) filled with 19 mL distilled water and kept at room temperature (approximately 100:1 water-to-leaf ratio). Leaf samples were taken from each tube every hour, and any excess water on the leaf surface was removed with tissue. Then, the leaf was weighed and returned to the tube. This procedure was repeated until the leaf weight remained constant. The experiment was done in triplicates.

The longest rehydration time among all treatments was used as a rehydration time for further investigations (electrical conductivity and photosynthesis) to ensure that every leaf sample was rehydrated to its maximum rehydration capacity and for the same amount of time.

2.5.4. Conductivity

When rehydration was completed, the electric conductivity of the rehydration water was measured with a conductometer at 21 °C (Orion Research Inc., Jacksonville, FL, USA). The leaf samples were returned to their rehydration tubes until the photosynthesis and respiration tests were performed (not longer than 30 min). The conductivity of the samples was compared to the conductivity of leaves that were frozen at –18 °C for 30 min and thawed at room temperature for 1 h.

2.5.5. Photosynthesis and respiration

The photosynthesis and respiration rates of leaf samples were determined using a modification of the method described in Panarese et al. (2014) [15]. For 20 min prior to the measurement, the leaf samples were kept in darkness. At a temperature of 20 °C, measurements were performed with an oxygen electrode (S1 O_2 electrode, Hansatech, Norfolk, UK) equipped with a thermostated electrode chamber (LD2/3, Gas-Phase Oxygen Electrode Chamber) and an integrated light source producing 380 μmol m^{-2} s^{-1} (LS3 Computer Controlled UV Light Source, Hansatech, Norfolk, UK). Thai basil leaf samples were cut into leaf discs measuring 3.5 cm in diameter and placed on a fabric plate soaked in bicarbonate buffer with a pH of 9. The buffer was prepared by one part of 0.4 M $NaBO_3$-buffer (pH 9) and two parts of 1 M Na_2CO_3-buffer (pH 9). The measurement began with a 10 min dark respiration measurement (light turned off), followed by a 10 min photosynthesis measurement (light on). The oxygen generation (for photosynthesis) and oxygen consumption (for respiration) were expressed as nmol(O_2) min-1 cm-2. Prior to measurement, the O_2 electrodes were calibrated with air and N_2. Seven rehydrated leaves were randomly selected from each drying procedure and their photosynthesis and respiration rates were determined.

2.5.6. Color

The color of fresh and rehydrated Thai basil leaf samples for every treatment was determined using a portable spectrophotometer (CM-700d, Konica Minolta, Konica Minolta Sensing Europe B.V.) with 10° standard observer and D65 light source with white plate calibration. An 8 mm width MAV target mask, which is suitable for color measurement of surfaces with uneven color was used. The measurement was performed perpendicularly to the sample and avoided the main vein on the leaves. Five measurements were performed on each sample. Numerical values of L*, a*, and b* CIELAB color space were used to obtain the total color change (ΔE, Eq. 2), which is a measurement of the color difference between fresh (f) and rehydrated (r) basil leaves.

$$\Delta E = \sqrt{\left(L_r^* - L_f^*\right)^2 + \left(a_r^* - a_f^*\right)^2 + \left(b_r^* - b_f^*\right)^2} \qquad (2)$$

2.5.7. High-resolution optical microscopy

Trichomes on the surface of Thai basil leaf samples dried with both methods to a MR of 0.05 and rehydrated to its maximum rehydration capacity were examined using an high-resolution optical digital microscope Keyence VHX-6000 (Keyence international (Belgium) NV/SA, Mechelen, Belgium) equipped with a diffuse light illumination.

Peripheral full-ring illumination was used along with digital glare-removal image processing. Micrographs were taken at 100X magnification (for trichome counts), 300X magnification (for surface quality investigation), and 700X magnification (for the measurement of area of the trichome). In a 2 mm^2 area (see **Figure** 1) of 10 leaves, the area covered by 3 individual trichomes was measured using tools integration in the microscope control software. In this way, a total of 30 trichomes were investigated for each treatment. The area covered by a trichome was calculated by drawing a baseline between each edge at the base of the trichomes in the 3D depth profile obtained from the software's "3D depth composition" function.

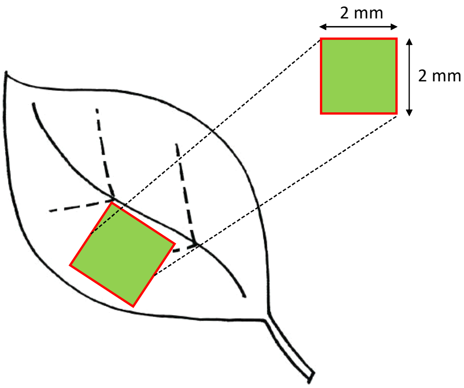

Figure 1 Investigated area on Thai basil leaf samples using high-accuracy digital microscopy.

2.5.8. Statistical Analysis

SPSS (v.26.0, IBM Corp., Armonk, NY, USA) was used to determine statistical significance (One-way ANOVA) at a significance level of 0.05. Tukey-HSD tests were used to conduct post-hoc analyses.

3. Results

3.1. Drying time, moisture ratio and water activity

Table 1 reports the experimental MR and water activity of Thai basil leaf samples that were dried to the target MR of 0.2, 0.1, and 0.05. Regardless of the pre-drying treatment, the water activity of the samples dried to the same MR were similar. For convective drying (CD), control-rested samples took longer to dry than control samples (relative drying time higher than 1), whereas PEF-rested samples took less time to dry than control samples (relative drying time less than 1) in all MR levels. Rested samples (both control and PEF-treated) required less vacuum drying (VR) time than control samples at all MR levels.

Table 1 Experimental drying time, moisture ratio (MR), and water activity (aw) of Thai basil leaves samples treated with different treatments (control, control-rested, and PEF-rested). Data are shown as average ± standard deviation of the mean for n=3 and different letters denote significant differences at $p<0.05$.

Treatments	Drying methods	Experimental drying time (min)	Relative drying time compare to control	Target MR	Experimental MR	aw*
Control	CD	62	Control (1.00)	0.2	0.191±0.027	0.841±0.034^{a}
Control-rested	CD	92	1.48	0.2	0.194±0.018	0.864±0.017^{a}
PEF-rested	CD	49	0.79	0.2	0.212±0.019	0.823±0.034^{a}
Control	CD	123	Control (1.00)	0.1	0.113±0.008	0.614±0.013^{b}
Control-rested	CD	205	1.66	0.1	0.091±0.013	0.605±0.013^{b}
PEF-rested	CD	95	0.77	0.1	0.103±0.018	0.612±0.016^{b}
Control	CD	204	Control (1.00)	0.05	0.049±0.008	0.465±0.040^{c}
Control-rested	CD	368	1.81	0.05	0.046±0.004	0.512±0.054^{c}
PEF-rested	CD	154	0.76	0.05	0.052±0.005	0.478±0.035^{c}
Control	VD	110	Control (1.00)	0.2	0.185±0.006	0.807±0.004^{a}
Control-rested	VD	60	0.55	0.2	0.204±0.024	0.849±0.040^{a}
PEF-rested	VD	40	0.36	0.2	0.202±0.030	0.811±0.008^{a}
Control	VD	390	Control (1.00)	0.1	0.094±0.007	0.630±0.031^{b}
Control-rested	VD	110	0.28	0.1	0.109±0.018	0.617±0.030^{b}
PEF-rested	VD	90	0.23	0.1	0.109±0.014	0.604±0.010^{b}
Control	VD	672	Control (1.00)	0.05	0.045±0.004	0.494±0.049^{c}
Control-rested	VD	652	0.97	0.05	0.049±0.006	0.509±0.043^{c}
PEF-rested	VD	592	0.88	0.05	0.054±0.005	0.491±0.046^{c}

*different letter superscript in each column indicates statistically significant differences ($p < 0.05$).

3.2. Rehydration capacity

Table 2 reports the maximum rehydration capacity (RC) of Thai basil leaf samples dried to the moisture ratios of 0.2, 0.1, and 0.05 using CD and VD. At MR of 0.2 and 0.1, the convective dried, rested samples (control and PEF-treated) show the highest RC. This effect of pre-treatments on RC is lost upon complete dehydration (MR = 0.05). Among the vacuum dried samples, resting shows to influence RC only at MR of 0.2. **Figure** 2 shows the rehydration curves of PEF-rested samples (treatment with the highest RC) dried to MR levels of 0.2, 0.1, and 0.05. At the MR of 0.2 and 0.1, CD samples reached the maximum rehydration capacity faster than VD samples. There were no differences in the rehydration time to the maximum rehydration capacity of the samples at MR of 0.05 for either drying procedures.

Table 2 The maximum rehydration capacity (RC) obtained after 18 h rehydration of Thai basil leaf samples subjected to different pre-treatments and dried by convective drying (CD) and vacuum drying (VD) to MR of 0.2, 0.1, and 0.05. Data are shown as average ± standard deviation of the mean for n=3

Pre-treatments	Drying methods	RC (kg water/kg dry matter) *		
		MR = 0.20	MR = 0.10	MR = 0.05
Control	CD	8.05 ± 0.33[b]	4.50 ± 0.20[a]	2.81 ± 0.39[a]
Control-rested	CD	9.17 ± 1.99[bc]	5.35 ± 0.05[b]	3.52 ± 0.51[a]
PEF-rested	CD	11.6 ± 0.78[c]	6.28 ±0.29[c]	3.59 ± 0.57[a]
Control	VD	6.33 ± 0.07[a]	4.22 ± 0.22[a]	2.79 ± 0.61[a]
Control-rested	VD	7.59 ± 0.99[b]	4.21 ± 0.07[a]	3.58 ± 0.39[a]
PEF-rested	VD	7.61 ± 0.19[b]	4.24 ± 0.12[a]	3.43 ± 0.58[a]

**different letter superscript in each column indicates statistically significant differences (p < 0.05).*

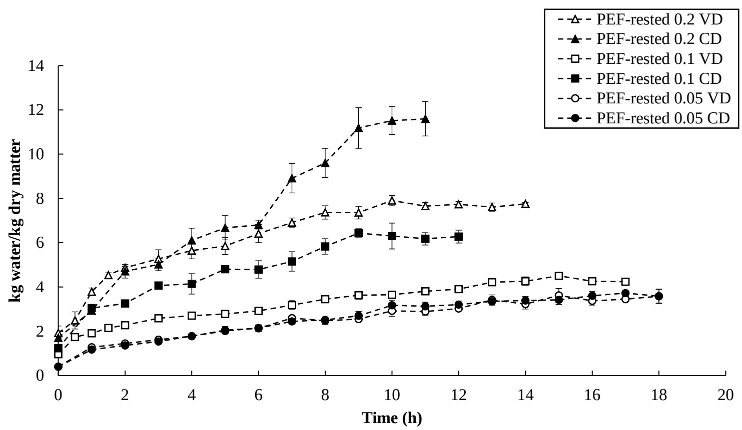

Figure 2 Rehydration curves of PEF-rested Thai basil leaf samples dried with convective drying (closed symbols) and vacuum drying (open symbols). Samples were dried to the MR of 0.2 (triangles), 0.1 (squares), and 0.05 (circles). Reported are average values of 3 measurements. Error bars represent the standard deviation of the mean.

3.3. Ion release during rehydration

The amount of ions released during the rehydration process increases the electrical conductivity of the rehydration water. Conductivity results for each pre-treatment, drying method, and moisture ratios are shown in **Figure** 3A-C. Samples that were convectively dried had lower electrical conductivity than those that were vacuum dried for moisture ratios of 0.2 and 0.1 ($p < 0.05$). At these levels of MR, the PEF-rested, convective dried samples showed the lowest values of conductivity, whereas the conductivity of the vacuum dried samples were equal or similar to that of the sample that was frozen and thawed. There was no difference in conductivity between the samples after drying to an MR level of 0.05 (**Figure** 3C).

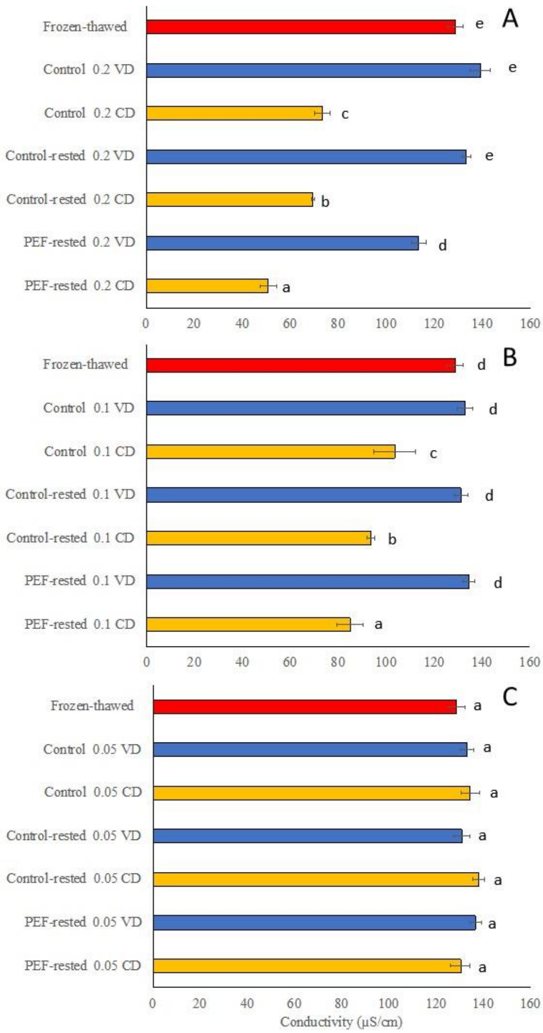

Figure 3. Electrical conductivity of rehydration water of dried Thai basil leaf samples subjected to different pre-treatments (Control, Control-rested, and PEF-rested) and dried with two methods: convective drying (CD) and vacuum drying (VD) to the moisture ratio of 0.2 (A), 0.1 (B), and 0.05 (C). Reported are average and standard deviation of 21 measurements. Different letters next to the error bars indicate statistically significant differences ($p < 0.05$).

3.4. Photosynthesis and respiration

In comparison to convective dried samples, vacuum dried samples at MR 0.2 had lower oxygen generation and consumption rates ($p < 0.05$) (**Figure** 4). At the MR of 0.1, convective dried samples did not show any photosynthesis capability, whereas vacuum dried, rested samples (both control and PEF) showed a slight photosynthesis activity at these MR levels (in the range of 0.48-1.61 % of that of the fresh sample). At this level of MR, the oxygen consumption rate of the PEF-rested, convective dried samples was significantly higher than that of the vacuum dried samples ($p < 0.05$). At MR of 0.05, neither respiration nor photosynthesis were detectable, irrespective of the drying method used.

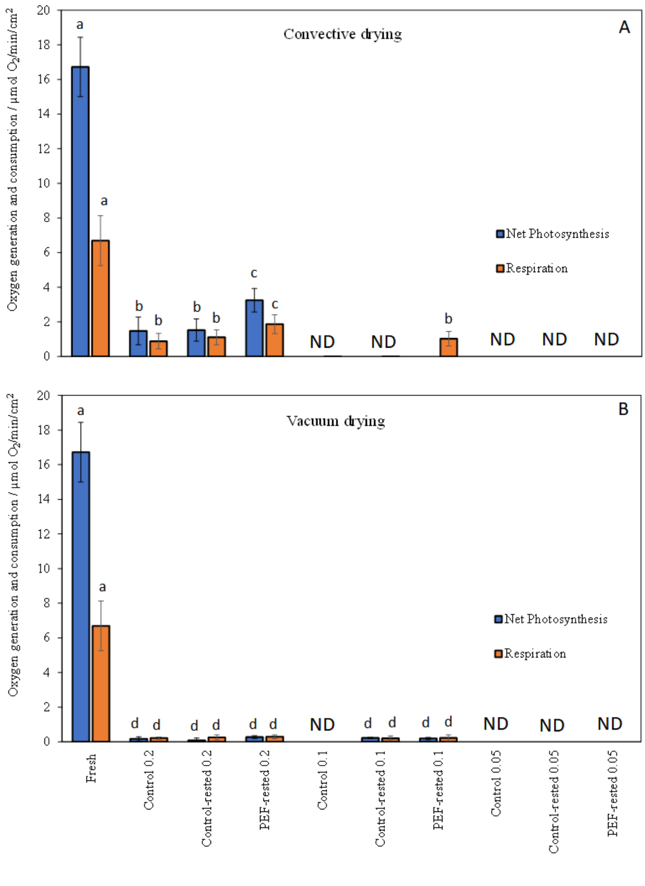

Figure 4. Oxygen generation and consumption of rehydrated Thai basil leaf samples subjected to different pre-treatments (control, control-rested, and PEF-rested) prior drying using convective or vacuum drying. The measurements were done with light source on (photosynthesis), and light source off (respiration). N.D.: not detectable. Reported are average and standard deviation from 21 measurements.

3.5. Leaf color

The total color change of rehydrated Thai basil leaf samples for all pre-treatments and drying methods are shown in **Figure** 5. The color change is significantly higher when convective drying is used at every level of MR. Dehydrating from MR 0.2 to MR 0.1 did not have significant effect on color changes. However, complete dehydration to MR 0.05 significantly increased the color change for both drying methods.

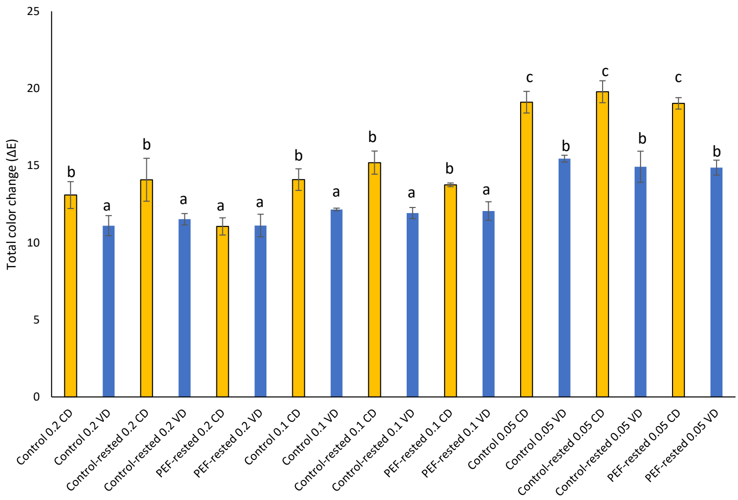

Figure 5 Total color change (ΔE) of Thai basil leaf samples subjected to different pre-treatments (control, control-rested, and PEF rested) prior drying with convective drying and vacuum drying to the moisture ratio of 0.2, 0.1, and 0.05. Reported are averages and standard deviations of 15 measurements. Different letter superscripts indicate statistically significant differences ($p < 0.05$).

3.6. Trichome structure

The number of trichomes on the leaf surface was between 5-7 trichomes/2 mm2 area of the leaf surface (square area, **Figure** 6A). **Figure** 6B-H shows representative micrographs of trichomes on the surface of Thai basil leaf samples at a magnification of 300X. Fresh samples (**Figure** 6B) showed a more intact structure than rehydrated samples (**Figure** 6B-H) . PEF-rested samples had less collapsed surface in both convective (**Figure** 6E) and vacuum drying (**Figure** 6F) than the rehydrated control and control-rested treated samples (**Figure** 6G and H).

When examined under a magnification of 700X, intact trichomes can be generally seen as expanded structures sticking out from the surface of the leaves (**Figure**. 7A). Partially collapsed trichomes were also found on the fresh samples (**Figure** 7B), but in very small numbers (less than 5%). Rehydrated samples showed different kind of trichomes:

intact (**Figure** 7C), partially collapsed (**Figure** 7D), and collapsed trichomes (**Figure** 7E). Intact trichomes can be seen more in fresh samples than in the rehydrated samples. When the area covered by a trichome was measured (**Figure** 7F), the area in fresh samples and PEF-rested, convective dried samples were found to be the largest (**Table** 3). In both convective and vacuum drying, the percentage of collapsed trichomes in PEF-rested samples was significantly lower than in control and control-rested samples ($p < 0.05$). In these samples, the area of partially collapsed trichomes were between 78-97% of the intact trichomes.

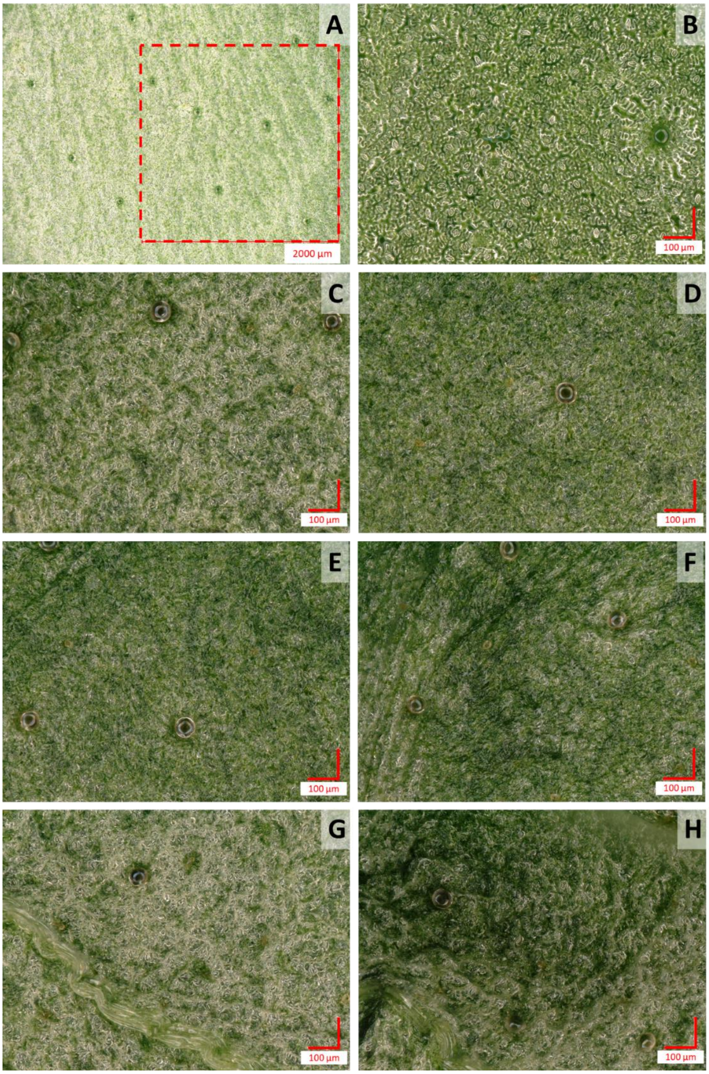

Figure. 6 Representative micrographs of fresh and rehydrated Thai basil leaf samples subjected to various treatments and examined using a high-resolution optical microscope: micrograph showing the number of trichomes in a 2 mm2area (A) with a magnification during acquisition of 100X, fresh (B), convective dried control (C), vacuum dried control (D), convective dried, PEF-rested (E), vacuum dried, PEF-rested (F), convective dried, control-rested (G), vacuum dried, control-rested (H). The magnification of the micrographs B-H during acquisition was 300X.

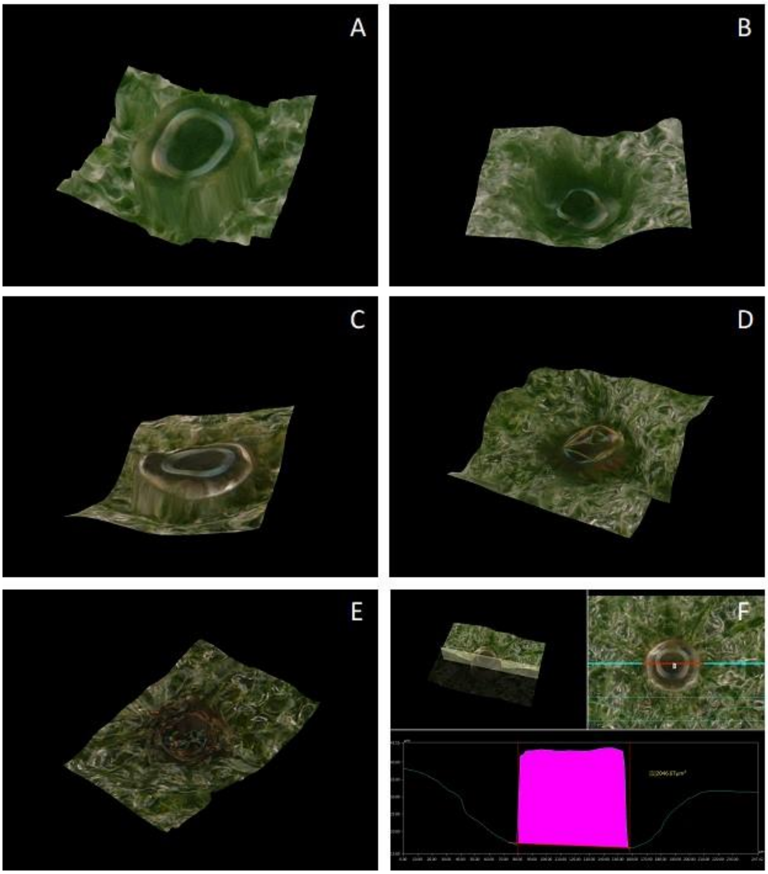

Figure 7 Representative 3-D surface images of different levels of intact and damaged trichomes found in fresh and rehydrated Thai basil leaves. Fully inflated trichome in fresh samples (A), damaged trichome in fresh samples (B), inflated trichome found in rehydrated samples (C), partially inflated trichome in rehydrated samples (D), damaged trichomes in rehydrated samples (E), schematic of trichome area measurement using the built-in software of the microscope, the measurement area shows the value of 2046.67 μm^2 (F). The magnification of the image during acquisition was 700X.

Table 3. Microscopic evaluation of trichome areas of Thai basil leaves subjected to different pre-treatments prior to drying with convective air (CD) at 40 ºC or under vacuum (VC). The samples were dried to an MR of 0.05. Data are shown as average ± standard deviation of the mean for n=3

Samples	Drying method	Partially collapsed trichomes (%)	Collapsed trichomes (%)	Area of trichomes (µm2)
Fresh	CD	3 ± 2^{a}	0 ± 0^{a}	2267 ± 89^{a}
Control	CD	33 ± 5^{b}	18 ± 4^{cd}	1204 ± 133^{cd}
Control-rested	CD	27 ± 3^{c}	19 ± 5^{cd}	1001 ± 115^{cde}
PEF-rested	CD	20 ± 4^{d}	5 ± 3^{b}	2218 ± 65^{a}
Control	VD	32 ± 5^{b}	23 ± 3^{d}	727 ± 80^{e}
Control-rested	VD	29 ± 4^{c}	15 ± 3^{c}	827 ± 102de
PEF-rested	VD	27 ± 5^{c}	7 ± 3^{b}	1785 ± 76^{b}

4. Discussion

Reversible electroporation can reduce the drying time of Thai basil leaves when used as pre-treatment for both convective and vacuum drying (**Table** 1). The faster drying rate induced by the reversible electroporation and guard cells electroporation was previously reported by Kwao et al (2016) and Telfser and Gómez Galindo (2019)[4, 8]. In this investigation, the PEF-treated samples were allowed to rest for 24 h in darkness before drying. In both convective and vacuum drying, the PEF-rested samples had a faster drying time than controls. This result can be explained by the stomatal opening caused by guard cell electroporation, which persists during resting[13].

When comparing the properties of the vacuum dried and convective dried samples dried to MR 0.2 and 0.1, it can be seen that the vacuum dried samples have a lower rehydration capacity (**Table** 2), higher release of ions to the rehydration water (**Figure** 3) and lower respiration and photosynthesis capacities than convective dried samples (**Figure** 4) for all pre-treatments, demonstrating that vacuum drying caused more tissue collapse and damage to the cells, leading to increased cell death.

In convective drying, a constant drying rate occurs as the food's internal moisture migrates outward to the surface at the same mass transportation rate as the moisture evaporation at the food's surface. This allows the internal moisture to remain liquid until the surface moisture has mostly evaporated [16]. As a result, the main damage to the cells may be caused by the heat of the drying process, in which the protective effect of resting may be able to protect the cells at certain levels of dehydration. Vacuum drying, on the other hand, exposes food tissues to a very low vapor pressure, resulting in extreme vapor pressure difference between the food tissues and the atmosphere, which lowers the boiling point of the moisture in the food tissues [17]. As a result, the internal moisture of food tissues evaporates directly as vapor from the food structure. Therefore, vacuum drying may cause more damage to the cells compared to convective drying.

Our results also show that the effect of resting after PEF treatment on protecting the cells upon certain levels of dehydration, described in Thamkaew et al (2021)14 and confirmed with our results (**Table** 2, **Figures** 3 and 4), is limited to high water activities and could not be seen upon complete dehydration, where cell damage was comparable between the PEF-rested samples and the ones that were frozen and thawed (**Figure** 3).

Remarkably, the PEF-rested samples dried with either method show more intact trichomes than the control-rested samples and the area covered by each trichome was similar to that in fresh leaves, though mainly for convective dried samples at 40 ºC (**Table** 3). This is a surprising result, as it could be expected that collapse and cell damage would occur at every level of the tissue upon complete dehydration. It seems that resting after reversible PEF treatment still exerts a protective effect on the leaf surface, including the trichomes.

Leaf trichomes play a crucial protective role against several biotic and abiotic plant stress factors[18] as they are directly exposed to the surroundings, often first encountering challenging environmental conditions[19]. Therefore, trichomes can act as a component of physical defense against stress and their morphology and secretory properties may adapt accordingly. Abiotic stress conditions such as UV irradiation has been reported to increase the concentration of polyphenols, including lignin deposition in sharp-headed trichomes[20]. PEF-induced morphological or structural changes on trichomes is an interesting issue for further research as trichomes contain the most characteristic aroma compounds of basil leaves, which may also be better preserved or even increased during the time of resting as a consequence of PEF-induced tissue perturbation[21].

5. Conclusions

The following are the paper's primary findings:

- Under the studied drying conditions, vacuum drying provoked more cell damage and tissue collapse than convective air drying at MR of 0.2 and 0.1. Under complete dehydration (aw = 0.05) damage seems to be similar for both drying methods irrespective of if the leaves were PEF pre-treated or not.

- The protective effect of resting after reversible PEF application on metabolic and ionic integrity was only detected at high water activities, being suppressed upon full dehydration (aw = 0.5).

- Samples dried under vacuum showed less color degradation upon rehydration when compared to convective dried samples.

- When dried with either convective and vacuum drying, reversible electroporation followed by resting resulted in higher trichome preservation than the samples that were not PEF-treated. When the PEF-rested treatment was combined with convective drying, the area of the trichomes was found to be similar to that of the fresh leaf sample.

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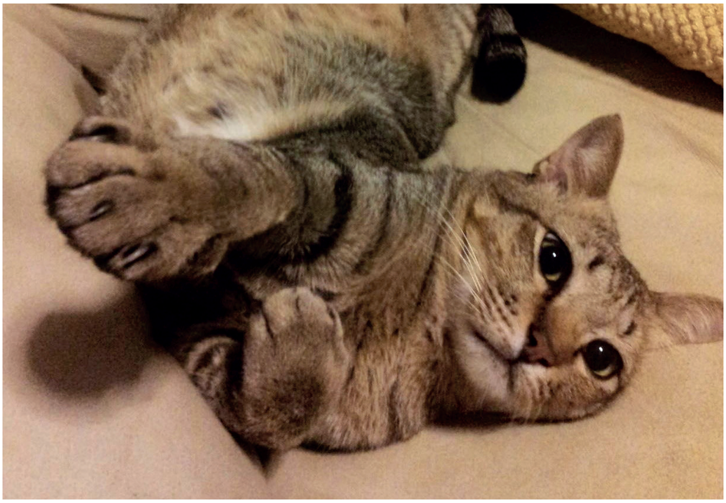

Have you ever seen this cat?

HIS NAME IS LONGGONG. He's a wonderful cat! Unfortunately, you can't see him any longer, neither do I.

To achieve one's life goals, one must make sacrifices. Many things have been sacrificed in order for me to finish my PhD, one of which is an opportunity. When my dear friend Longgong died, I missed the opportunity to be with him in his last moment.

Before starting this Ph.D. journey, I believed that the way to be successful in life was to gathering things that others admired. I spent far too much time pursuing degrees, careers, money, and those superficial things, while leaving countless invaluable behind.

Longgong's death taught me that it doesn't matter how far I've come in my life. What beside me at this very moment determines my life. There is no point in trying to have everything in the world only to be left with no one to share the joy at the end of the road.

Success isn't defined by what you have received,
but what you've been able to maintain.

I learned it too late for Longgong. I'm not going to be late for anything else in my life.

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