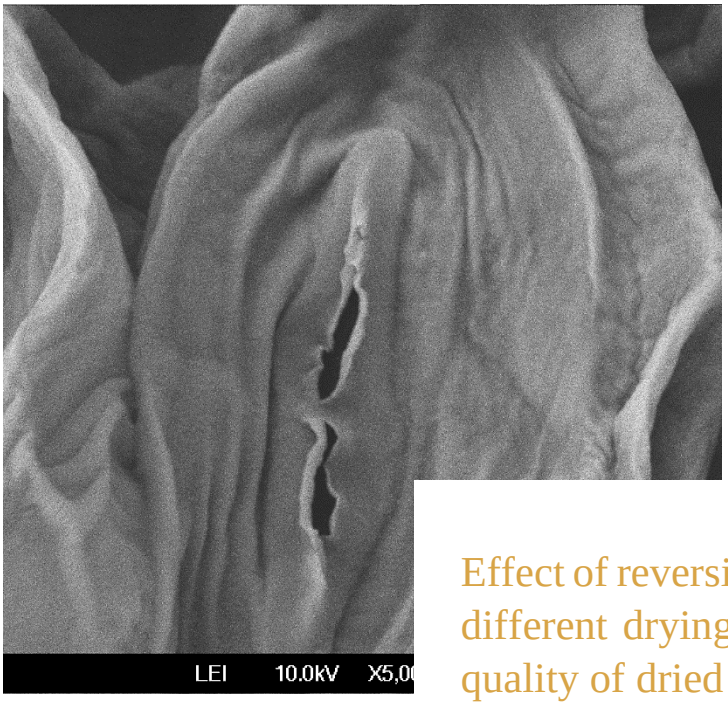

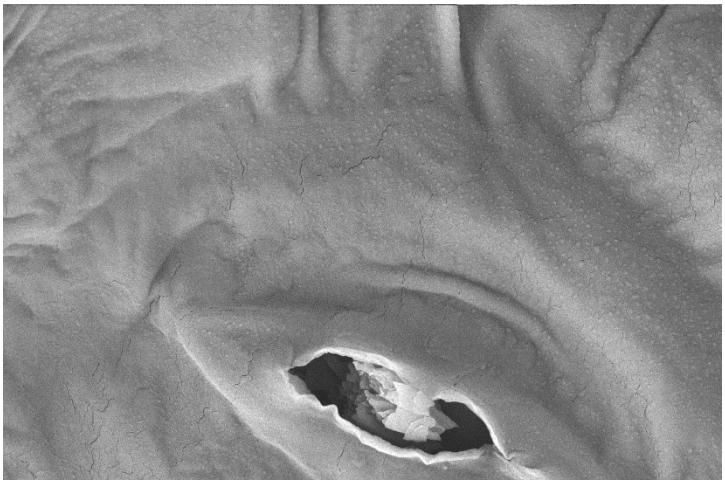

Effect of reversible permeabilization in combination with different drying methods on the structure and sensorial quality of dried basil leaves

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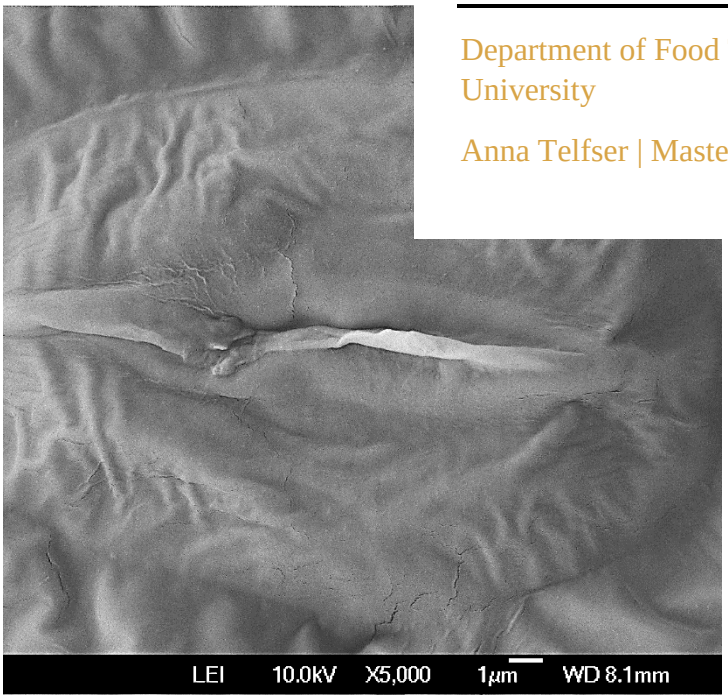
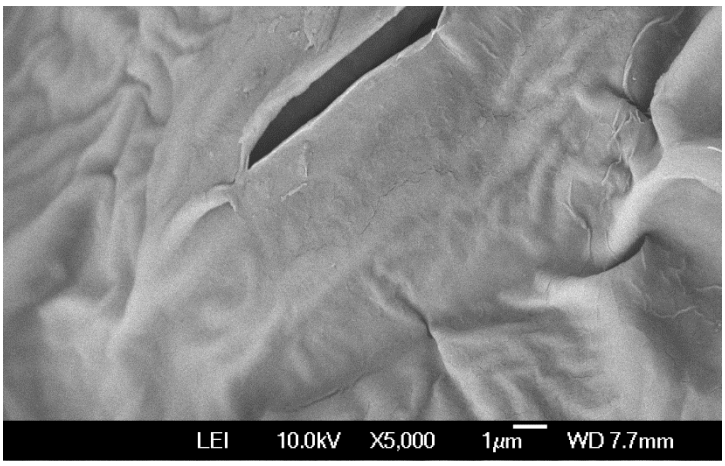
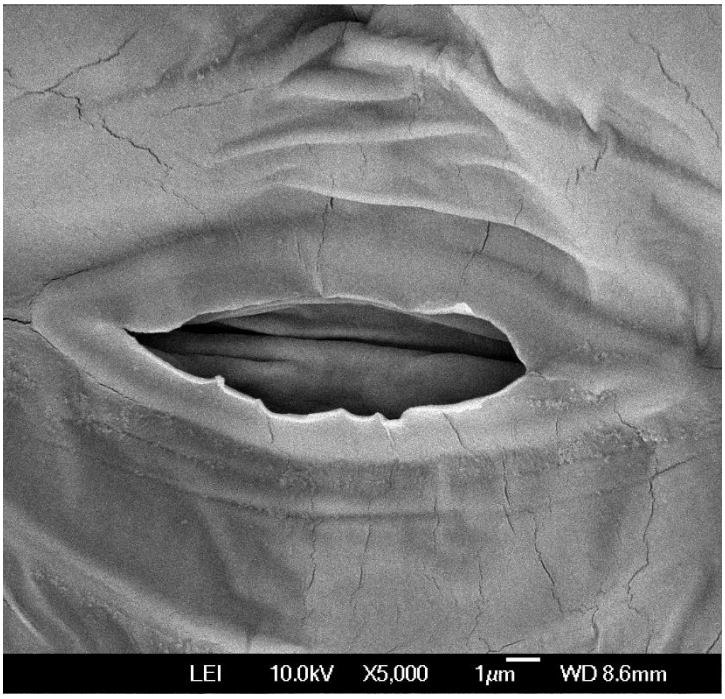
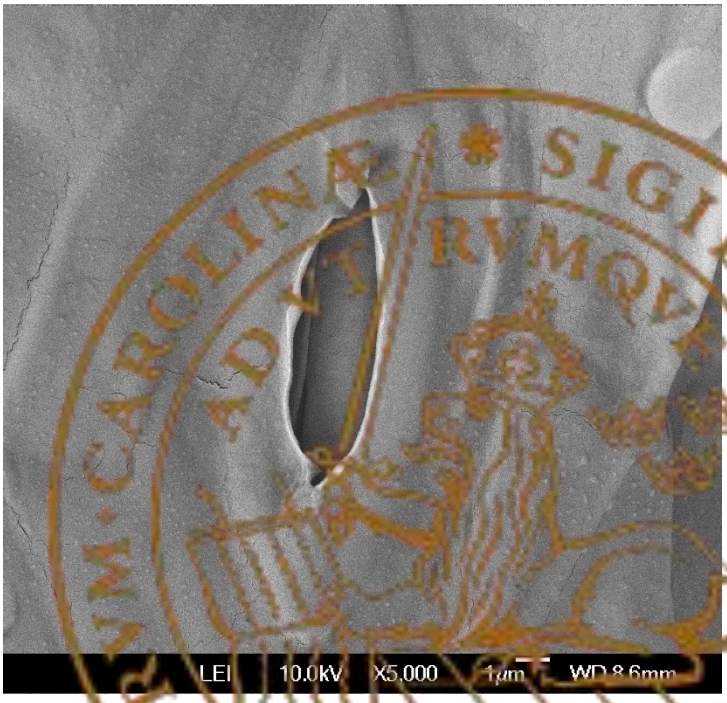
Anna Telfser | Master Thesis in the field of Food Engineering









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Lunds Tekniska Högskola

Effect of reversible permeabilization in combination with different drying methods on the structure and sensorial quality of dried basil leaves

Anna Telfser

Master of Science Thesis in Food Technology and Nutrition 2017

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Abstract

In this work the impact of different drying techniques and the stomata aperture induced by pulsed electric field (PEF) prior drying on quality of the dried products was assessed. The conditions of the electroporation treatment were chosen to induce an irreversible opening of the stomata of the basil leaves. The drying times of the different drying techniques used (air-, vacuum- and freeze drying) were adjusted to obtained products with a moisture content of about 7.5 % and a water activity between of about 0.3-0.5. Dried samples were compared according to sensorial quality, the degree in cell collapse, the degree in color changes and the rehydration capacity.

The results showed that the quality properties of dried basil were influenced by both, drying technique and the irreversible stomata aperture induced by pulsed electric field treatment.

The different drying techniques and the PEF treatment showed an influence on drying time, where the PEF treatment reduced the drying time needed to reach the same amount of moisture content.

The degree of cell collapse was assessed with scanning electron microscopy. It was shown that the choice of drying technique influences the degree of cell collapse, with freeze dried samples showing the best-preserved cell structure. The PEF treatment influenced the degree of cell collapse with an exception in freeze dried samples, which were not different from each other. In contrast the rehydration capacity assessment did not show differences depending on the drying technique or PEF treatment between the samples.

The preservation of the aromatic oil cavities, containing the aroma compound were assessed with SEM and showed that there was no influence of aroma regarding different drying techniques observed. PEF treatment significantly enhanced the aroma compound retention, with an exception of the freeze dried samples.

When assessing the aroma compounds through the sensory evaluation, there was an influence on aroma compound retention between different techniques and PEF pre-treatments. The PEF treated basil were the samples with more preserved aroma compounds. An influence of the drying technique used according to aroma compound retention was perceived, where for a dried basil and pesto the vacuum dried samples were shown to retained most aroma compounds, as assessed by sensory evaluation.

An influence of the different drying techniques on the color change after drying was observed. Air dried samples resulted in a yellower green product when compared the fresh sample and the vacuum and freeze dried samples changed towards a darker green. The PEF treatment showed a slight influence in the color change, but to a much lesser extent than the drying techniques. The sensory panel detected clear color differences of the air dried samples compared to the samples dried with the other techniques and a rather clear difference between the untreated and PEF treated air dried sample. However, differences between untreated and PEF treated sample of the other two techniques were difficult to detect.

The industrial implementation of PEF treatment prior to drying was suggested for air- and vacuum drying and was found to be inadvisable to freeze drying.

Table of Contents

List of Abbreviations

AD	Air dried
ANOVA	Analysis of variance
C	Chroma
CIE	Commision Internationale de l'Eclairage
D_{eff}	Effective moisture diffusivity
ESEM	Environmental electron microscopy
FD	Freeze dried
FDA	Fluorescein Diacetate
h	Hue angle
k	Drying constant
M_e	Equilibrium Moisture content
M_0	Initial Moisture content
M_t	Moisture content at any time "t" during the drying process
MR	Moisture ratio
PEF	Pulsed electric field
PI	Propidium iodide
R^2	Coefficient of determination
RC	Rehydration capacity
RMSE	Root mean square error
RT	Room temperature
SEM	Scanning electron microscopy
SSE	Sum of square error
t	Drying time
VD	Vacuum dried

1 Introduction

Sweet basil is an aromatic herb (Paton, Harley and Harley, 1999), widely used for providing a unique aroma for many applications, such as in food, medicine and perfumery products (Putievsky and Galambosi, 1999). It is commonly used in its dry form, as shelf life of the harvested leaves can be significantly increased when applying a drying step post-harvest (Mäkinen and Pääkkönen, 1999).

Nowadays the production of high quality dried herbs is gaining more importance, as the competition on the market is large and the demand is increasing, because people are used to cook with herbs throughout the whole year even if out of season. Sensorial attributes of the product should be kept as close as possible to the fresh standard, where it is known that different drying techniques result in products providing specific sensorial characteristics (Prothon, *et al.,* 2003). Therefore, the investigation of the drying impact of different techniques on the product is crucial to achieve a high-quality product.

Ideally, a high quality dried products should not show full cell collapse, should retain the entire cell wall and keep the intracellular space intact. High, rehydration capacity is a parameter indicating the preservation of the cell structure during drying (Prothon *et al.,* 2003). Additionally, during drying the reduction in aroma compounds should be kept at a minimum (Mäkinen and Pääkkönen, 1999) and the color change occurring should be minimized. The color change is a result of the degradation of chlorophyll a and b, carotenoid pigments and browning reactions (Mäkinen and Pääkkönen, 1999).

An increase in quality of air dried basil was observed when applying a pulsed electric field (PEF) treatment to the basil before drying (Kwao *et al.,* 2016). This treatment is based on the theory that the water evaporation in leaves takes place through small openings, the so-called stomata, where the opening and closing of the stomata helps the plant to regulate excessive water loss. The opening and closing of the stomata is steered by the turgor pressure within the so-called guard cells, which surround the stomata. During the drying process the stomata will close to protect the leaves from desiccation (Cosgrove and Holbrook, 2010).

The PEF treatment applies short electric impulses to the basil leaves through a conductive solution. This pre-treatment was shown to reversible electroporate the cells of the leaves creating small holes in the cell membrane which will be able to reclose during the drying process again keeping the cells viable (reversible permeabilization) (Dymek *et al.,* 2014). This reversible permeabilization causes a structural change in the guard cells of the leaves keeping the stomata irreversibly open, through which the water evaporates during the drying process. The PEF treatment resulted in lower drying times, hence less heat impact on the basil leaves creating a product with higher quality parameters than the untreated control. (Kwao *et al.,* 2016) An investigation on the influence of reversible permeabilization in combination with different drying methods on structure and sensory characteristics could provide information needed to produce high quality dried basil.

2 Objectives

2.1 Aim

The aim of this work was to examine the influence of PEF-induced stomata aperture in combination with different drying techniques on the aroma sensorial perception, structure, color changes and the rehydration capacity of dried basil leaves.

2.2 Detailed objectives

Detailed objectives were set to be:

- To determine PEF conditions to achieve a reversible permeabilization of cells, with irreversible opening of the stomata
- To identify drying times of untreated and PEF treated basil leaves with different drying techniques to achieve a product with similar moisture content
- To determine influence of stomata aperture on the drying times
- To investigate the influence of drying techniques and PEF treatment on the structure/cell collapse
- To determine the influence of drying techniques and PEF treatment on sensorial perception
- To investigate the influence of drying techniques and PEF treatment on color

3 Background

3.1 Basil plant

Sweet basil is an aromatic herb, often used culinarily in many food and beverage applications due to its distinct flavor (Paton, Harley and Harley, 1999) and it is known to have antioxidant effects (Hossain et al., 2010), but it is also used in the medical field (Paton, Harley and Harley, 1999) as it shows antibiotic properties (Putievsky and Galambosi, 1999).

Genovese basil makes part of the sweet basils (Ocimum basilicum L.), which belongs to the family of mint the so called Labiatae (Díaz-Maroto et. al., 2004). Ocimeae have their origin in tropical countries such as parts of America, Africa, India and Asia. (Paton, Harley and Harley, 1999) Nowadays the cultivation of basil is spread all over the world due to its high demand, where most of the basil produced is used for dried basil production, followed by essential oil production and lastly used for domestic consumption. The harvesting time of the basil leaves influences the amount and composition of essential oils present, as for example the leaf size will vary. It is hard to find a general standard for the best harvesting time, as it depends on the kind of basil used and where the basil is produced. (Putievsky and Galambosi, 1999)

3.1.1 Genetic related chemical composition

There are different cultivars of sweet basil (Ocimum basilicum L.), which show differences regarding chemical composition, chemotypes and the number of chromosomes (Paton, Harley and Harley, 1999). Cross pollination provides an increasing number in species of Ocimum, which show differences in morphology and chemical composition. This means that depending on the seed supplier, basil plants sold even if commercialized with the same name might have different characteristics. The identification of the chemotype of the industrially used basil plants is therefore common. (Putievsky and Galambosi, 1999) Ocimum basilicum was divided by researchers into 4 or 5 different chemotypes, where the principle is that the different subspecies are grouped according to the most abundant compounds constituting the essential oil. Commonly, they contain high amounts of linalool (30 - 90 %) and methyl chavicol (50 - 90 %), other important compounds are 1,8-cineole, eugenol and methyl eugenol, which can be present in free form or bound to sugar. The chemotype characterization has drawbacks, as it gives only an idea about the composition of the essential oil in the fresh leaves. When the leaves are dried some compounds decrease due to evaporation, while others accumulate, which could lead to a shift in essential oil composition, resulting in characteristics of a different chemotype after drying. (Hiltunen and Holm, 1999)

3.1.2 Use

The original use of basil back in the day was more superstitious than culinary. The word basilikos is translated from the Greek to royal, as in Greece only royals could harvest basil, but it symbolized trouble and hate. On the other hand basil belonged to traditional Hindu weddings and in Africa they believed that eating basil was aiding against any harm. In India it was a symbol for good luck and in Italy and Russia it was a plant for lovers. (Mäkinen and Pääkkönen, 1999)

Nowadays basil is a widely used herb as it provides a unique aroma for many applications. It can be either used in its fresh form, or after processing it in frozen or dried form, or as essential oil.

- Fresh basil: Basil in its fresh form is used to contribute its distinct flavor to foods and beverages, such as sauces, stews, aromatic oils and liqueurs. It also used for decorative purposes in restaurants.
- Frozen basil: With the exception of their decorative use, the frozen basil is used for the same purposes as the fresh leaves. It has an advantage that its availability does not depend on the growing season of the plants.
- Dried Basil: Postharvest dried basil is the most abundant form of basil leaves used for culinary reasons giving flavor to several dishes, especially to tomato containing dishes.
- Basil Oil: Essential oil extracted from basil finds its use as well in food industries, being used in the production of sweets, bakery products, processed meat and liqueurs. Not only food industries use basil oil, it plays a role in perfumery, including perfumes and toiletries.
- Medicinal use: There is the general believe that basil can be used for medicinal purposes, helping for example against headache, cough or gut irritation. It was also reported to be used for veterinary purposes, as the essential oil is showing antibiotic characteristics. (Putievsky and Galambosi, 1999)

3.1.3 Structure

To understand what happens to a basil leaf during drying it is important to know the cell structure, with a focus on the water locations and interactions, to be able to get an idea what structural changes might the cell undergo when the water evaporates during drying.
A basil leaf consists of several organized epidermal cells, where the cell wall and membrane enclose a nucleus, the cytoplasm and several cell organelles (Wolniak *et al.*, 2010). The cells keep their shape due to turgor pressure within them, which is a pressure of the water filled vacuoles on the cell walls kept constant by osmosis. These cells build up a tissue called parenchyma, which stores water and nutrients. It is scattered with gas filled regions and vascular tissue. (Prothon *et al.*, 2003)

3.1.4 Physiology/water loss during drying

The water transfer out of the basil leaves is conducted through stomatal pores, by diffusion of water vapor due to a difference in concentration of water vapor between the inside of the leaves and its surroundings. The stomata are normally open during daytime and closed during nighttime, as photosynthesis occurs during daytime. Only then CO_2 can be up taken and water can evaporate as new water is introduced into the system through the roots and brought to the leaves. To reach the stomatal pores, the movement of water within the tissue is triggered by a difference in water potential within the cells. A reduction in osmotic potential within the guard cells, hence a rising osmotic pressure is induced due to the intake of ions through the shift of solutes from surrounding cells and because of biosynthesis. This provokes a reduction in water potential and water is up taken, which leads to an increase in guard cell volume and a higher turgor pressure, while reducing the volume and turgor pressure of neighboring cells, causing the opening of the stomatal pore. The regulation of the water loss depends on two additional factors, where one is the leaf stomatal resistance, which is highest when the stomata are closed and lowest when they are open. The other one is the boundary layer resistance, which is shown during the diffusion of the water vapor through still standing air that coats the leaf, until it reaches the circulating air of the environment. This resistance depends on the thickness of the air coat, which again depends on the size of the leaves and on the pace of the circulating air of the environment. It means that the boundary layer resistance will be low if the air circulation around the leaves is fast, causing the leaf stomatal resistance to be the main defense mechanism of the leaves against desiccation. (Cosgrove and Holbrook, 2010)

3.1.5 Defense mechanism against extensive water loss

The main task of a basil leaf is photosynthesis, which is important for the plant to grow and reproduce cells. Plants are naturally prone to dehydration by continuous water evaporation throughout the whole day, therefore they evolved a technique to avoid extensive water loss (Wolniak *et al.*, 2010). On one hand, they have an almost water impermeable waxy cuticle which covers the whole leaf, preventing the evaporation of most of the water contained. On the other hand, the leaves have stomatal pores which can regulate the amount of water leaving the leaf. The opening and closing of the stomata is conducted to regulate the turgor pressure in so called guard cells, where two kidney shaped guard cells enclose one stomatal pore (see Figure 1). (Cosgrove and Holbrook, 2010)

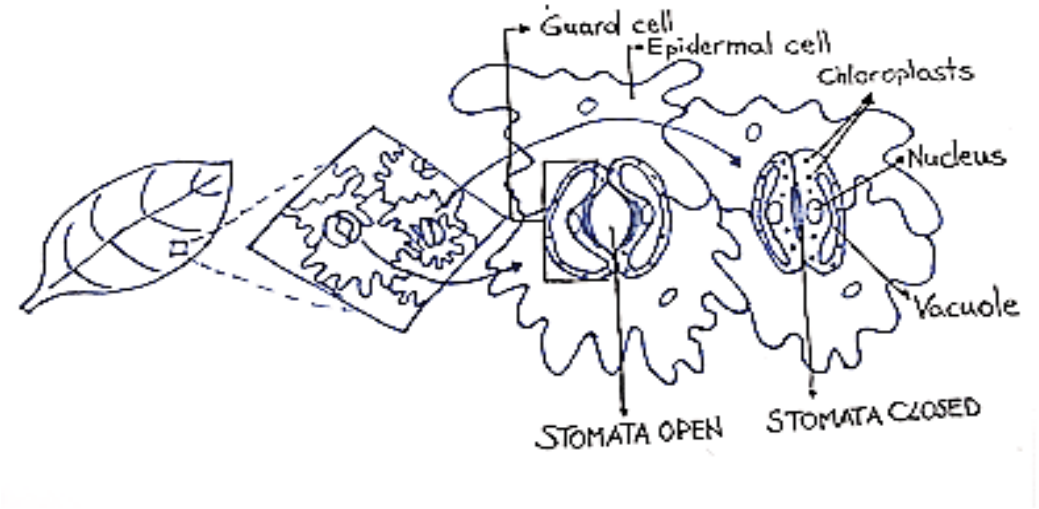

Figure 1 Epidermal cells surrounding guard cells with open and closed stomata

Guard cells are characterized by a thicker inner cell wall and a thinner outer wall. This facilitates the opening of the stomata if the vacuoles get filled with water, expanding the cell volume and so bending the outer walls towards the surrounding epidermal cells. Not only the amount of water in the leaves provoke the opening of the stomatal pore, there are several environmental aspects involved, such as the presence of light and the amount of CO_2 present in the cells. (Cosgrove and Holbrook, 2010)

3.1.6 Storage of essential oils

As mentioned before, the essential oils produced by the basil plant are important for many applications. These essential oils are produced by so called glandular trichomes, which are hair like extensions of the epidermis of the basil leaves. Basil leaves contain peltate trichomes (see Figure 2), which are made from a basal cell, a petite stalk cell and a head with numerous cells secreting the essential oils. The head is leading to a cavity, formed by the detachment of a membrane of the secretory cells, which stores the essential oils. Once this cavity is filled with the essential oils it will result in a round, bulb like form.

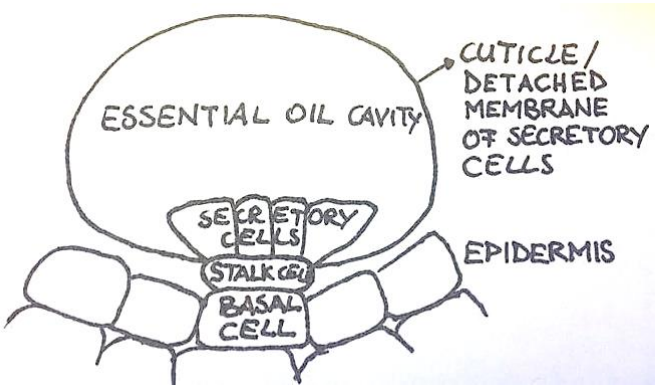

Figure 2 Glandular trichome filled with essential oils

The essential oils produced by the glandular trichomes are normally used by the plant as a defense mechanism against pathogens and plant parasites. (Glas *et al.*, 2012) But the essential oils were not only found to be important for the plants itself, but also for industrial reasons, as mentioned in section 3.1.2.

3.2 Drying

3.2.1 Pre-treatment (Pulsed electric fields)

Pulsed electric fields (PEF) is a non-thermal technology most commonly used for prolongation of the shelf life of liquid foods, but it was as well reported to be used for boosting juice extractions and to strengthen the drying process of some foods. It induces short pulses of electricity with high voltage to material positioned between two electrodes. At very high voltages microorganisms are killed, while bacterial spores resist the treatment. Most studies about PEF processing were conducted with liquid or semi liquid products, because it is a good method for preserving foods with high water activities by disabling microorganisms, while minimizing changes in their sensorial and nutritional attributes avoiding heat treatment. (Mohamed and Eissa, 2012)

PEF treatment of basil leaves

The concept of PEF is based on the fact that a liquid containing ions possess a certain electrical conductivity, so that the electrical pulses applied can be shifted to every point in the liquid. (Mohamed and Eissa, 2012) When implementing PEF treatment as a pre-treatment for drying techniques, the idea is to achieve structural changes of the guard cells to keep the stomata open for a faster drying process (Kwao, *et al.*, 2016). Depending on the parameters chosen for the electric pulses, it is possible to kill the cells (irreversible permeabilization) or to keep the cells viable (reversible permeabilization) (Dymek *et al.*, 2014). The reversible form keeps the cells viable as they are able to recover once the created pores in the cell membrane close again, but the stomata open irreversibly, resulting in a shorter drying time and better quality characteristics of the dried product (Kwao *et al.*, 2016). There are several parameters which play a role in the electroporation to obtain a reversible permeabilization, such as pulse polarity, pulse duration, pulse frequency, cell size, and their distribution and orientation. (Dymek *et al.*, 2014)

3.2.2 Drying techniques

Drying is a postharvest process, which is applied for a better preservation and so to prolong the shelf life of the basil leaves, as they are highly perishable. The aim is to achieve a reduction in moisture content to at least 10 % (to a water activity < 0.6), to guarantee a safe product. (Hiltunen and Holm, 1999)

During the drying process, there is a shift of mass and heat between the product and the environment. The difference in temperature between the surrounding and the sample surface triggers the movement of heat, at the same time the difference in moisture between the inside and the surface of the sample lead to the shift in mass. (Prothon *et al.*, 2003)

Moisture will be removed from the food product, which can be bound or unbound water. The evaporation of the unbound water, which is the water on the surface of the material, takes place rather easily. The bound water is trapped within the solid food system and this water must travel within the food towards the surface for evaporation. The rate of evaporation of the unbound water depends on the temperature of the environment, the air flow, the humidity, the size of surface area and the pressure. The evaporation rate of the bound water depends on the product and its moisture content throughout the drying process. (Parikh, 2015)

During drying the leaf parenchyma loses water. There are three different steps to describe the water movement during the drying process:

- Wall-to-wall transport is the capillary movement of water via the cell walls. It is mostly used for water transport in living tissue.
- Cell-to-cell transport is the movement of water via vacuoles, the cytoplasm and the cell membrane. This is the mode of transport that predominates in the initial phase of drying when the moisture content is high.
- Intercellular transport is the flow of water vapor via the intracellular space. As the moisture content decreases, this mode of transport starts to predominate.

As the water will be transported with a phase change out of the food, pores might be created which finally collapse due to a loss in turgor pressure, resulting in a loss of the cell structure. (Prothon *et al.*, 2003)

Air drying

There are two drying systems most commonly used, the spontaneous air drying, and the artificial drying with the help of forced heated air. The first one is mostly applied in developing countries due to money saving reasons leading to an uncontrolled drying process prone to potential contamination. The second system is used in industrial production, where the processes are controlled, but varying machineries might be used, such as conveyor driers, or plate-chamber driers. (Putievsky and Galambosi, 1999)

Convective hot air drying is the most commonly used technique in the industry, but it does not have a high energy efficiency as the drying times are rather long compared to other drying techniques. Additionally, it leads to changes in sensorial characteristics of the food product, such as color differences and nutrient decrease. (Orikasa *et al.*, 2013.)

During commercial air drying it is important to know the exact drying time for a specific product to obtain a final product with a certain moisture content. Mathematical modeling is often used to design specific drying curves for new products. When designing a drying curve for agricultural products, thin layer drying equations are commonly used. (Ademiluyi and Abowei, 2013) Several thin layer drying equations exist, which can be fit to experimentally obtained data. During the drying process of a new product, it is necessary to monitor the moisture content, air velocity and temperature at a constant relative humidity along a certain time span to elaborate a drying curve. (Aregbesola *et al.*, 2015)

Dimensionless moisture ratio

By monitoring the moisture content of a product over time during experimental trials, the dimensionless moisture ratio (MR) can be calculated with Equation 1.

$$Moisture\ ratio = \frac{M_t - M_e}{M_0 - M_e} = \frac{M_t}{M_0} \quad (1)$$

where M_t corresponds to the moisture content at time "t" (kg water/kg dry matter), M_0 corresponds to the initial moisture content (at "t" = 0) (kg water/kg dry matter) and M_e corresponds to the equilibrium moisture (kg water/kg dry matter), which can be neglected due to long drying times. During long drying times M_e is significantly smaller by contrast with M_t/M_0, so that the equation can be simplified as shown in Equation 1. (Sadi and Meziane, 2015)

Newton model

A curve where MR is plotted versus the drying time of the product is normally elaborated, where each product shows a specific drying curve at set parameters. Once this curve is obtained, a thin layer drying equation can be fit to the data. The most simplified model is the Newton model, it assumes that there is no resistance during the moisture transferred within the product, the only resistance encountered is on the surface of the food product. This model is displayed in Equation 2.

$$MR = \frac{M_t}{M_0} = \exp(-k * t) \quad (2)$$

where k corresponds to the drying constant (min^{-1}) and t corresponds to the time of drying. (Aregbesola *et al.*, 2015)

Logarithmic model

Another thin layer drying equation commonly fit to experimental data of agricultural products is the logarithmic model. The logarithmic model showed to be the most accurate model to estimate the drying kinetics of basil leaves. It derives from another thin layer equation, the Henderson and Pabis model and it includes three unknown constants, as can be seen in equation 3.

$$MR = \frac{M_t}{M_0} = a * \exp(-k * t) + c \quad (3)$$

where a and c correspond to dimensionless model constants. (Onwude *et al.*, 2016)

Effective moisture diffusivity

Not only the drying curve is individual for every product, also the effective moisture diffusivity (D_{eff}) is specific for a product. This means that an effective diffusion coefficient must be calculated for every product and every drying time. The mass transfer that takes place during the drying process can be explained by the movement of moisture during drying, thus, moisture diffusivity is a very important factor to consider during the modelling of the drying process. This movement of moisture during drying is a result of the difference in moisture content within and on the surface of a product, where the rate of mass movement is proportional to the difference in moisture content. (Vasić *et al.*, 2012)

The correlation between MR and D_{eff} can be seen in equation 4. In this equation, it is assumed that the movement of moisture takes place due to diffusion, that there is no shrinkage occurring during drying and that the temperature and the diffusion coefficient are constant throughout the drying process.

$$\ln MR = \ln \frac{M_t}{M_0} = ln \frac{8}{\pi^2} - \left(\frac{\pi^2 D_{eff} t}{4H^2}\right) \quad (4)$$

where H corresponds to half of the height (m) of the food product (a slab in the case of leaves), and D_{eff} corresponds to the effective diffusivity (m^2/s). (Aregbesola *et al.*, 2015)

To predict the effective diffusion, it is possible to plot ln(MR) versus time, which results in a slope, that can be used to calculate the value for the effective diffusion coefficient with the following equation. (Aregbesola *et al.*, 2015)

$$slope = \frac{\pi^2 D_{eff}}{4H^2} \quad (5)$$

Vacuum drying

Reduced pressure is used to increase the speed of drying while keeping the temperature low (at room temperature) (Orikasa *et al.*, 2013). In this way energy is saved, which is cost efficient and shows environmental advantages (Parikh, 2015). As the drying occurs without the presence of air, no oxidation takes place during the process, preserving the sensorial properties of the product (Orikasa *et al.*, 2013).

Vacuum drying is not often used for vegetables, as the equipment is costly and samples must be dried batch wise (Prothon et al., 2003).

Freeze drying

Freeze drying is a technique that avoids heat damage of the product, but is rather costly (Di Cesare et al., 2003).

Freeze drying can be described in 3 phases:

1. Freezing of the product. The water present in the product freezes and an ice crystal structure is formed. (Parikh, 2015)

2. Primary sublimation, in which the vapor pressure of the ice must be higher than the vapor pressure of the environment (Parikh, 2015). This makes sure that the ice crystals formed during former freezing of the product will sublimate. (Prothon *et al.*, 2003)

3. Secondary sublimation, where the remaining water will be removed. Here the lowest vapor pressure of the product throughout the drying process will be achieved. (Parikh, 2015)

During freeze drying the ice crystals formed in the freezing step will be sublimated and replaced by vacuum and later by air. This creates new pores in addition to the already existing in the food structure, therefore, the final product will be highly porous. (Prothon *et al.*, 2003).

3.2.3 Drying impact on basil quality characteristics

Drying has a positive effect on the shelf life of basil, as it decreases the water activity lowering the risk for growth of microbes and slowing down biochemical processes. However, drying has unwanted impacts, on the quality of the product such as changing the color, taste and smell. To reduce the extent of these unwanted changes, the drying temperatures in conventional air drying should be kept as low as possible, where it is recommended to dry the basil leaves at 40°C to keep the reduction of quality as low as possible (Mäkinen and Pääkkönen, 1999).

Aroma

To retain the aroma compounds during the drying process within the product is one of the main goals of producers, as the taste and smell of the product is the reason why consumers buy it.

Aroma compounds can be volatile or nonvolatile, where the volatile ones are very delicate when heat treated during the drying process. This means that the drying process can cause a decrease in aroma compounds. (Mäkinen and Pääkkönen, 1999)

Color

The perceived change in color after the drying process is a result of the decomposition of chlorophyll a and b, carotenoid pigments and browning reactions (Mäkinen and Pääkkönen, 1999). Where chlorophyll a (blue green colored) and b (yellow green colored) are losing their bound Magnesium (Mg^{2+}) during the drying process shifting from a bright green color to an olive brown color. As chlorophyll a is more unstable during heat treatment it will be more easily degraded, shifting the color of the dried product more in direction yellow green. Additionally, enzymes are favoring the breakdown of chlorophyll through another pathway creating a product (chlorophyllides), which are more prone to lose Mg^{2+}. The brown color formation during drying can be explained by the breakdown of the cells during drying (cell collapse, which will be discussed later), releasing the chlorophyll and acidic compounds, which react with each other to create pheophytin, a product prone to enzymatic browning. (Di Cesare et al., 2003)

When the basil leaves are dried the reduction in moisture content induces a modification in the cell shape, volume and the osmotic functionality. If cells are dying during the drying process, they lose the ability of controlling the permeation of salts and water, so with a loss in turgor pressure they lose the crispy texture and due to a reduction in moisture content the typical rubbery texture is transformed into a brittle and glassy one. Further, there is a modification in the crystallinity of the cell wall observed. (Prothon *et al.*, 2003)

Texture

Cell collapse is happening during the drying process, as the water evaporates pores are created, therefore the turgor pressure cannot be built up anymore, which causes the cells to collapse upon themselves. This phenomenon can only be called collapse if it is resulting in an unrepairable shrinkage of the food. Water molecules are normally attached by hydrogen bonds to the phospholipids in the lipid bilayer of the cell membrane, to which additional water molecules can bind rising the strength of the membrane. During drying these bonds will break, as water evaporates, where sugars could replace the water molecules, but if only a small amount of sugar is present, the cells undergo collapse, changing the plant texture as described above. (Prothon *et al.*, 2003) In the early drying stages the volume of shrinkage is equal to the volume of water lost, later in the drying process this correlation cannot be made anymore (Lewicki *et al.*, 1997). The extent of shrinkage of a food and the resistance of it against this deformation is dependent on the type of food and the pretreatment applied to it. Further it was shown that different drying techniques result in different outcomes when looking at the collapse and shrinkage of the cells. For freeze dried samples shrinkage is only observed if the drying temperature is higher than the glass transition temperature of the basil leaves, which is during freeze drying not the case. However, a bulk shrinkage happens for all tissues. Further freeze dried leaves were found to have an about 50 % higher porosity than air dried ones as after the sublimation of the ice crystals big pores remain, which are stabilized in their structure, avoiding cell collapse. Air dried samples undergo a high extent of shrinkage, which decreases the amount and volume of pores. These structural changes Resulted in diverse rehydration abilities and different extents of retaining aroma compounds within the structure. (Prothon, *et al.*, 2003)

The exact cause for collapse is not yet understood, there are several theories for the reason of collapse during drying. Some state that the glassy state of the vegetable tissue created during

drying and its soluble parts (mono- and disaccharides) play a main role. Others state that the cell wall structure plays the main role. In a few theories, the pressure induced by the capillary action is suggested to cause pore collapse. In another theory is explained that the water evaporation creates a pressure that pulls in the cell walls. (Prothon *et al.*, 2003)

3.2.4 Quality assessment

Sensorial evaluation

To examine the sensorial properties of a product a sensory evaluation is commonly used. During a sensory evaluation, the quality characteristics of a product will be assessed, where not only the taste and smell of the product will be examined, but also every other perception that the product provides through senses such as color and texture. There are two categories of sensory evaluation, (i) objective testing, in which a chosen or trained panel assesses the quality characteristics and (ii) subjective testing, where random consumers examine the product. In industries, sensory evaluation is not only used to study the sensorial characteristics of a product, but also to gain understanding about consumer acceptance and consumer behavior. (Kemp *et al.*, 2009)

A commonly used tool for objective testing are focus group discussions, with are profound interviews with a small group of panelists. This tool is used in many fields as a qualitative research approach. It is favorable to gain not only better understanding about the product to be assessed, but also additional information for example about their consumption histories, or about the marketing aspects such as the consumers perception of prices and brands. Further the interaction of the group members is thought to deliver more data compared to individual interviews. A disadvantage of the focus group is that the group dynamic and answers might be influenced by panelists with strong personalities. (Stewart *et al.*, 2007)

Color and color change examination

The color of a product can be examined by spectrophotometric analysis, which measure the color pigments of the leaf, (Sass *et al.*, 2012) giving information about the average color of the measured area (Frausto-Reyes *et al.*, 2009). The measured color correspond to the chemical compounds present in the food which transmit color (Wrolstad *et al.*, 2005). Colorimetric analysis helps to describe a color with numerical values, as the description of a color with human eye is highly complex (Frausto-Reyes *et al.*, 2009).

Nowadays there are numerous standards set to describe complex color systems, which were defined by the Commision Internationale de l'Eclairage (CIE). One of them is the CIE *L*a*b** that describes three parameters indicating the color of a product. These parameters are correlated to the perception of color by the human eye. (Frausto-Reyes *et al.*, 2009) As seen in Figure 3, the L* value, gives an idea about the lightness of the product where 0 indicates black and 100 indicated white. The a* value indicates the color either towards greenness if negative, or towards redness if positive. The b* value indicates the color either towards the blueness if negative or towards yellowness if positive. (Wrolstad *et al.*, 2005)

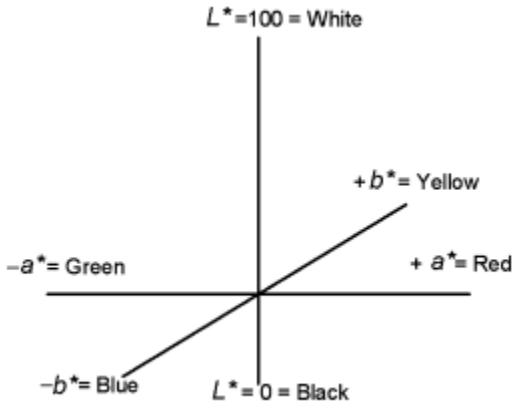

Figure 3 CIE *L*a*b** axis (from Witt, 2007)

With the help of these parameters the total color change (ΔE) can be obtained (Frausto-Reyes *et al.*, 2009). A value of ΔE^*_{ab} of about 2,3 means that a just noticeable difference can be observed with the naked eye (Wikipedia, 2017).

It is important to keep in mind that the color is three dimensional; a better way of describing it is with the help of CIE *L*C*h* system. Where the L*value was already described before and the Hue angle (h_{ab}) can be obtained from the a* and b* values of the sample. As seen in Figure 4, the Hue angle is described on the base of a 360° lattice, in which 0° describes colors from bluish to red, up to 90° there is a transition to yellow, from 90° going towards 180°C a green color is observed and 270° corresponds to blue. In this system, a difference in 1° can already be noticed with the naked eye. The saturation of the color indicated by the hue angel can be expressed by the chroma (C). If a sample shows the same hue angle, the chroma can indicate the intensity of the color, where a higher value for the chroma means a more intense color (see Figure 4). (Wrolstad *et al.*, 2005)

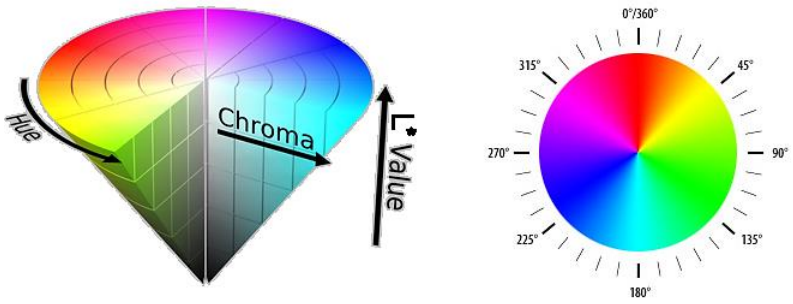

Figure 4 CIE *L*C*h* color system and the hue color wheel (adapted from Adobe Technical Guides, 2000 and Wikimedia Commons, 2016)

Rehydration

The extent of rehydration gives information about the degree of tissue injury and the remaining textural properties after drying. It was shown that with a lower extent of cell collapse a better rehydration capacity was achieved. (Prothon *et al.*, 2003)

During rehydration, the water is transported through cavities towards the tissue where it starts to enclose trapped air bubbles. Afterwards water diffuses through the amorphous regions of the solid phase, created during the drying process. The glassy part of the structure is modified into a rubbery structure, creating the ability for polymers to move, resulting in textural collapse

of the pores as the enclosed air holding the structure can escape. Therefore, at the beginning of rehydration, a reduction in size of the samples is observed, while after the collapse mobilized polymers can absorb water and the pores get filled with water, leading to an increase in volume. (Lewicki *et al.,* 1997)

Krokida *et al.* (1999) showed that no matter which drying technique was used, after rehydration a change in viscoelastic properties was observed, as the plant tissue underwent structural modifications during drying forming glassy regions. It was seen that the samples changed from more elastic behaviors towards rather viscous ones, with freeze dried samples showing the biggest changes.
During freeze drying structural changes might not be visible by eye, but there are microstructural modifications occurring, which upon rehydration lead to the formation of a soft and loose texture, typically observed when cells are injured. This damage takes place due to the ice crystal formation during freezing, where the ice crystal size and the ice formation rate determine the extent of injury. (Prothon *et al.,* 2003)

Structural examination

Textural modifications can be investigated with the help of microscopy, both after drying and rehydration. During scanning electron microscope (SEM) examinations, only dried products can be observed due to the need of low pressure in the microscope chamber which would cause dehydration of the sample rather fast, altering its cell structure. Additionally, fixation of the sample is needed using a metal coat to be conductive (Pathan *et al.*, 2008). A primary electron beam generated by the SEM is scanning the conductive surface of the sample, creating signals, which are collected by a detector, creating an image from the information obtained. There are two types of electron signals common during SEM, backscattered electrons and secondary electrons. The SEM pictures are created due to a difference in the intensity of the electron signal detected as the electron beam is scanning over different cell structures. (Stokes, 2008)

The original structure of a sample, at the hydrated state can be examined with an environmental scanning electron microscope (ESEM), which is a specialized microscope not commonly found in every laboratory. With this equipment, fast examinations of products with high moisture content, without additional sample preparation can be made, creating good pictures at low magnification. This is possible due to an option of adjusting vapor pressure in the chamber and the stage temperature on which the sample is placed. This means that the sample can be observed for longer time at high humidity. (Pathan *et al.,* 2008)

As in SEM, also here a primary electron beam scans the cell surface, but the secondary electrons are gaining speed in the area of the detector while colliding with the water vapor particles creating extra electrons. This leads to an intensification of signals received by a specific gaseous secondary electron detector, which balances the fact that the sample is not conductive. (Stabentheiner *et al.,* 2010)

The ESEM shows also limitations, as waxy microstructures on leaves cannot be observed and cell collapse might occur at high magnification. However, it provides the possibility to examine the native structure of plant tissues, which would not be possible with an ordinary SEM. Nowadays the ESEM examination gains more importance for the analysis of trichomes. (Pathan *et al.,* 2008)

4 Design of Experiments

In Figure 5 an outline of the experiments that were conducted throughout this study can be seen.

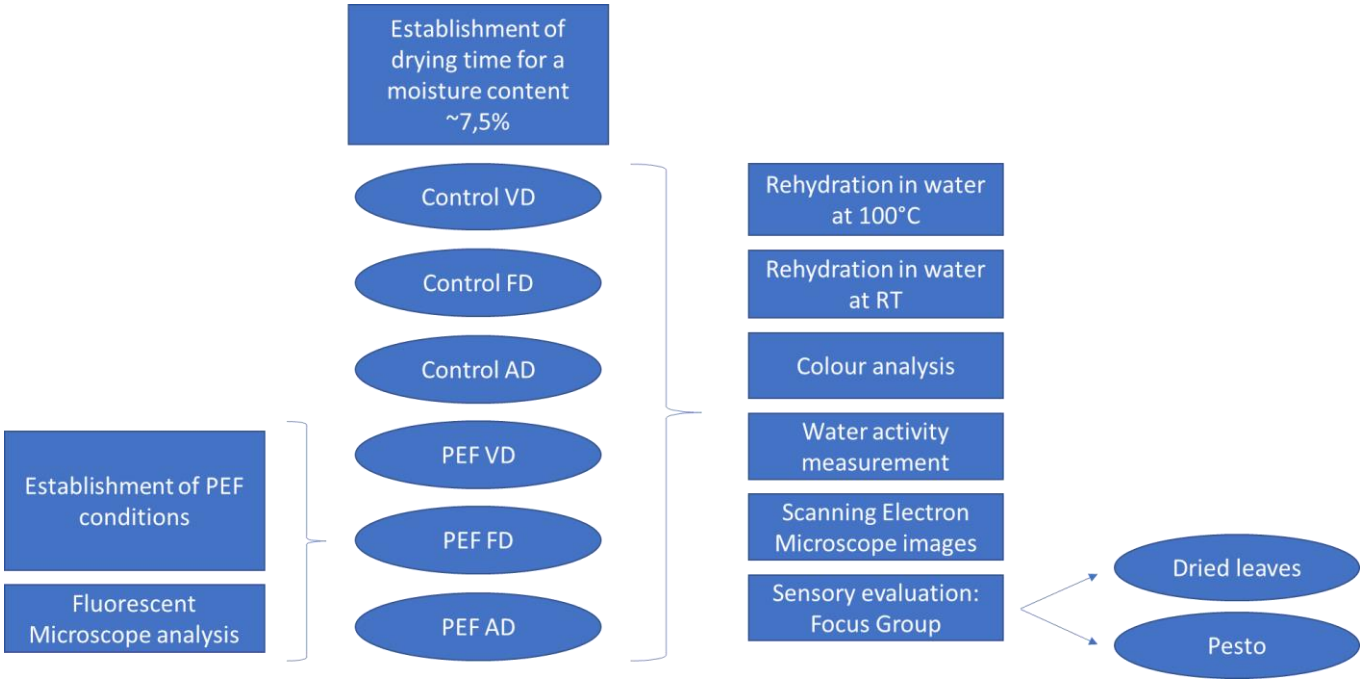

Figure 5 Outline of experiments, where PEF corresponds to pulsed electric field, VD corresponds to vacuum dried, FD corresponds to freeze dried and AD corresponds to air dried.

5 Materials and methods

5.1 Raw material

Ecologic potted basil (Ocimum basilicum L.) was obtained from a local farm (Kabbarps Trädgård AB, Åkarp, Sweden) and transported to the laboratory, shielding the plants from frost. The plants were kept for a maximum of one week at room temperature with constant light exposure until used for experiments.

5.2 Sample preparation before drying

Basil leaves 5 ± 0.5 cm length, 3.5 ± 0.3 cm width and a weight of 0.20 ± 0.05 g were harvested from the basil plant and directly used for processing.

5.3 Pulsed electric field (PEF)

The PEF treatment of the basil leaves was performed as described by Kwao *et al.* (2016) with slight modifications. Detached basil leaves were transferred into an electroporation chamber (see figure 6) which contained enough solution of deionized water and sodium chloride (NaCl) to cover both electrodes once the chamber was closed.

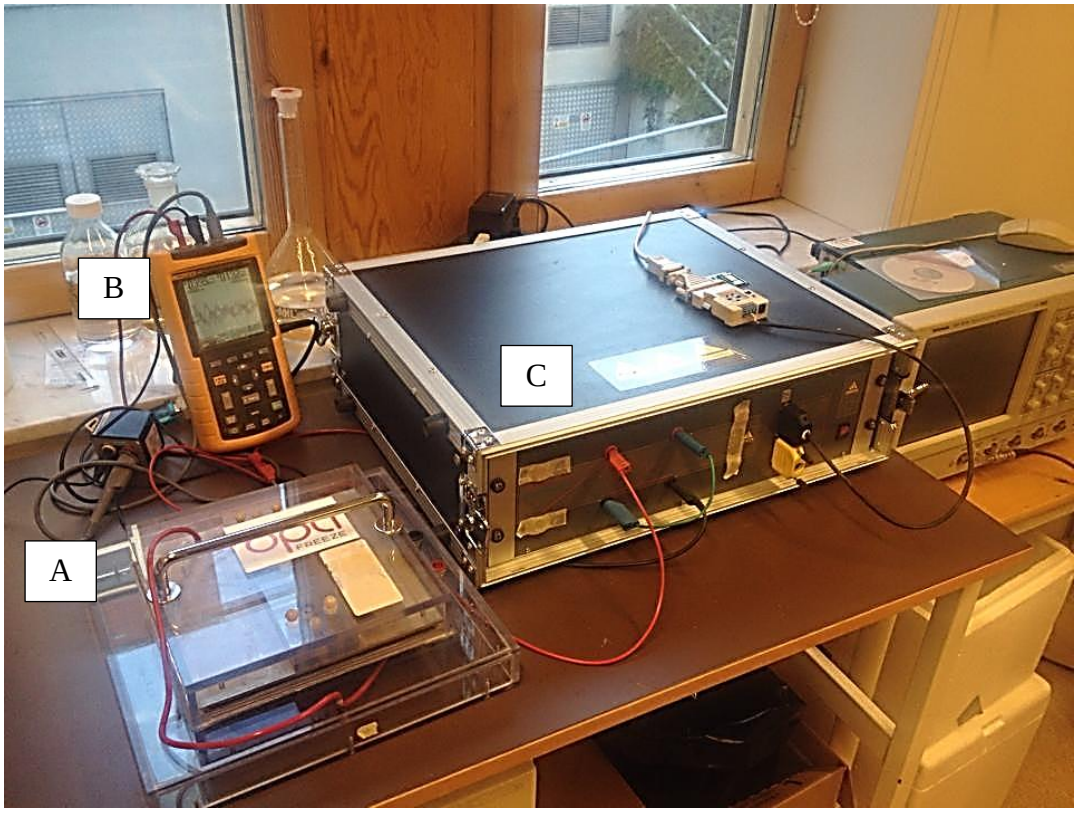

Figure 6 PEF-treatment setup: A) electroporation chamber (gap size between electrodes: 0.8 cm), B) oscilloscope, C) electric pulse generator

The salt solution was prepared by adding as much sodium chloride to deionized water to adjust the conductivity to 130 **µ**S. The conductivity was measured with an Orion conductivity meter (Jacksonville, FL, USA). The electrodes were connected to an ARC Aroma Pure AB CEPT® pulse generator (Lund, Sweden) where the electric pulses delivered could be monitored by a digital Fluke 123 oscilloscope (Washington, USA). The electric treatment of the basil leaves was operated with an ARC CEPT HM13 computer software (Lund, Sweden) where the conditions for the treatment were an electric field intensity of 650 V/cm, 150 **µ**s pulse width, 760 **µ**s pulse space and 65 pulses delivered in 1 train.

5.3.1 Fluorescent Microscopy for the evaluation of the PEF treatment

The electroporation of the guard cells due the PEF process was assessed as described by Dymek et al. (2014), with slight modifications. The basil leaves were placed in an electroporation chamber which was filled with enough conductive solution, in this case 300 **µ**M Propidium Iodide (PI) solution (130 **µ**S), to cover both electrodes when closed. After applying the electric pulses, the samples were washed with deionized water and examined under the fluorescent microscope with a red fluorescence filter ($\lambda_{ex} = 535$ nm, $\lambda_{em} = 617$ nm). A Nikon Eclipse Fluorescent Microscope Ti-U (Japan) was used with to identify red stained nuclei in electroporated cells.

The cell viability of basil leaves after PEF treatment was identified as described by Dymek *et al.* (2014), with slight modifications. The PEF treated basil leaves were stored at 5 °C for 20 h in a saturated environment, in a sealed container with a wet cloth. After storage, they were incubated in 12 **µ**M Fluorescein Diacetate (FDA) solution for one hour at room temperature in a dark room. The FDA solution was prepared by adding 200 **µ**L of a 12 mM FDA in acetone stock solution into 200 mL of MilliQ water. After the incubation in FDA the basil leaves were washed with deionized water and prepared for immediate examination under a Nikon Eclipse Ti-U Fluorescent Microscope (Japan) with a green fluorescence filter ($\lambda_{ex} = 492$ nm,$\lambda_{em} = 517$) at magnifications of x4 and x10.

Two control samples were prepared, (i) the positive control; a basil leaf that was freshly cut before incubation in the FDA solution and (ii) the negative control which was a PEF treated leaf, where the PEF conditions were adjusted to kill the cells of the leaf (650 v/cm, 400 **µ**s pulse width, 800 **µ**s pulse space, 990 pulses and 1 train). The storage conditions were the same as for the treated sample.

5.4 Drying methods

5.4.1 Air drying

Untreated basil leaves and basil leaves that underwent PEF treatment, with a sample size of 20.0 ± 0.5 g, were dried in a convection air drier at 40 °C with a constant airflow of 2 m/s. The leaves were evenly spread onto 3 trays which were placed on top of each other on an oven rack attached to a scale, where the scale was connected to a computer system (RS232 Monitor, EVM Software) that constantly recorded the samples weight loss over time. The experimentally recorded data was used to calculate the dimensionless moisture ratio (MR) with Equation 1.

When plotting the MR versus time a drying curve was obtained. A model fitting was used to calculate the drying time needed to obtain a final product with a moisture content of approximately 7.5 %. These leaves could then be used for further analysis.

5.4.2 Vacuum drying

Several trials were made to find the right time to vacuum dry batches of 20.0 ± 0.5g of untreated and PEF treated leaves at room temperature (18 °C - 21.5 °C) to obtain a moisture content of about 7.5 %. Untreated basil leaves were vacuum dried in a HETOSICC Freeze dryer (HETO BIRKER***Φ***D, Denmark), without operating the ice condenser, for an experimentally determined drying time with a final pressure of around 13 Pa. The PEF treated leaves were dried in the same equipment for an experimentally determined drying time with a final pressure of around 15 Pa.

5.4.3 Freeze drying

Sample batches of 20.0 ± 0.5g of untreated and PEF treated basil leaves were frozen in a BCF 10/5 Foster Blast Chiller (UK) at -30 °C. Freezing curves were obtained by plotting the temperature change over time. The data points were obtained by taping a thermocouple between two basil leaves and recording the temperature change over time. The average of four runs was used to create the freezing curves.

Trials were conducted to find the right time to freeze dry the frozen untreated and PEF treated basil leaves with an ice condenser temperature of around -50 to -40 °C, to obtain samples with a final moisture content of around 7.5 %. Untreated basil leaves were freeze dried in a HETOSICC Freeze dryer (HETO BIRKERΦD, Denmark) for an experimentally determined drying time with a final pressure of around 11 Pa. The PEF treated leaves were dried in the same equipment for an experimentally determined drying time with a final pressure of around 13 Pa.

5.5 Analysis

5.5.1 Moisture content

The moisture content of the fresh and the dried basil leaves was determined according to their weight loss upon drying, according to AOAC (1997) with slight modifications. Approximately 0.4 – 0.5 g of sample was weighed into a preheated and precooled dish, then they were heated in a Termaks drying oven (Bergen, Norway) at 104 °C and weighed after cooling in a desiccator. The samples were heated, cooled and weighed until constant weight was achieved. Equation 6 was used to calculate the percentage of moisture content in the sample.

$$Moisture\ content\ (\%) = \frac{(W_i - W_d)}{W_i} * 100 \qquad (6)$$

where W_i corresponds to the initial weight of the sample and W_d corresponds to the final constant dried weight.

5.5.2 Water activity

The water activity of the fresh basil leaves and all dried samples was measured with a CX-2 Aqua Lab water activity meter (Washington, USA).

5.5.3 SEM Examination

Sample preparation

Small rectangles, 5 x 3 mm were cut from the dried sample. Two pieces of each sample, one with the top surface of the leaf pointing upwards and one with the bottom of the leaf pointing upwards, were placed on one glue coated circular brass. The samples were coated with 15 nm of a gold and palladium mixture (80 % gold, 20 % palladium). This operation was done with a Blazers SCD 004 Sputter Coater, in argon atmosphere for 180 seconds at 5 - 10 mbar with an electric current of 15 mA.

Image acquisition

The conductive samples were placed in the sample holder, where 3 samples fit into one sample holder. After that samples were inserted into a field emission scanning electron microscope (JEOL, JSM-6700F, Tokyo, Japan) and the pictures were taken for each sample with the upright electron detector. This detector scatters a mixture of secondary electron beams and back scattered fields. The pictures were taken with an acceleration voltage of 10 kV at a chamber pressure of $9.63*10^{-5}$ Pa and a working distance of 7.5 - 8.6 mm.

Environmental scanning electron microscope examination of fresh basil leaves was made without any specific sample preparation. The ESEM pictures were taken in a scanning electron microscope (Zeiss, EVO LS 10, Germany), with an acceleration voltage of 10 kV at a chamber pressure of 70 Pa, a stage temperature of 1 °C and a chamber humidity of 10 %.

5.5.4 Rehydration

Rehydration at 100°C

One basil leaf of each sample was weighed and immersed in a beaker filled with 100 ml of boiling deionized water, removed, gently wiped to eliminate the excess water on the surface of the leaf and weighed again. Three replicates of rehydration measurements were made for each sample, where the data points recorded were the weights after 1, 2, 3, 4, 5, 10, 15, 20 and 30 minutes.

Rehydration at Room Temperature (~ 20 °C)

Three replicates of rehydration capacity examination were conducted, where for each sample one dried basil leaf at the time was weighed and afterwards immersed in a beaker with 100 ml of deionized water at room temperature. The leaf was removed after every hour, placed on and covered with tissue paper and gently patted for twenty times to eliminate excess water on the surface of the leaf. This procedure was repeated until observation of constant weight

For both rehydration experiments, the rehydration capacity (RC) was calculated with equation 7.

$$Rehydration\ capacity\ (\%) = \frac{(W_t - W_d)}{W_d} * 100 \qquad (7)$$

where W_t corresponds to the weight at time "t" and W_d corresponds to the initial dried weight of the basil leaf.

The rehydration capacity (RC %) was plotted against time for further comparison of the untreated and treated samples with the different drying techniques.

5.5.5 Color measurements

Sample preparation

The dried basil leaves were ground with an OBH Nordica coffee mill type 2393 (Sweden) for 30 seconds to produce a homogeneous powder for further color analysis and comparison to the fresh leaves. The fresh leaves were not ground before color measurements, as they displayed a uniform color.

Color analysis

The color of the fresh basil leaves and the ground dried samples was assessed with a spectrophotometer CM-700d from Konica Minolta Sensing, Inc (Japan). A MAV target mask with a 8mm width was used, which gives as an output an average value of the color detected in case of uneven colors. A clear plastic film was spanned between the target mask and the samples examined. Ten measurements of each sample were taken to obtain the values of L* (Lightness), a* (greenness) and b* (yellowness).

The total color change was calculated according to Equation 8 (Witt, 2007).

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \qquad (8)$$

L^*_1, a^*_1, b^*_1 correspond to the average values of the parameters of the fresh basil leaves

L^*_2, a^*_2, b^*_2 correspond to the average values of the parameters of the dried basil sample.

The hue angle (h_{ab}), difference in hue (Δh_{ab}), the chroma (C^*ab) and the difference in chroma (ΔC^*_{ab}) were calculated with the following equations (Schanda, 2007; Witt, 2007).

$$C^*_{ab} = (a^{*2} + b^{*2})^{1/2} \quad (9)$$

$$\Delta C^*_{ab} = C^*_2 - C^*_1 \quad (10)$$

$$h_{ab} = tan^{-1}\left(b^*/a^*\right) \quad (11)$$

$$\Delta h_{ab} = h_2 - h_1 \quad (12)$$

C_1 and h_1 corresponds to the calculated values of the fresh basil leaves,
C_2 and h_2 corresponds to the calculated values of the sample.

5.5.6 Sensory evaluation

Sample preparation

Dried basil leaves were ground by hand with a mortar to make the samples resemble the product in the market, where 0.5 g of sample was used for the sensory evaluation of the dried herbs and 0.8g of each sample was used to prepare 38.3 g of pesto for a second sensory evaluation. The pesto was prepared by processing the ingredients for in a Braun CombiMax 600 (Germany) at the highest speed for 1 minute. The recipes used for preparation of the fresh and the dried pesto can be found in the Appendix in section 12.1.

Focus group

Two focus group discussions were held to gain a better understanding of the perceived quality from costumers regarding dried basil and Genovese pesto. For the dried basil focus group a semi trained panel with 7 participants (4 males and 3 females) held the discussion and for the pesto a focus group with 6 semi trained panelists (3 males and 3 females) joined the discussion. The study question in these focus group discussions was to assess the sample that provided quality characteristics (with a focus on color, smell and taste) which were the closest to the fresh alternative.

Initially general information about the use and the most important quality aspects of dried basil and pesto were obtained from the sensory panel. After that a fresh basil sample and a commercial dried basil sample were given to the panel for examination of color, smell and taste to gain understanding of the characteristics of the two extremes.

Then a pair-wise comparison of the untreated and PEF treated samples dried with the same technique was made, where the samples were coded with random numbers of three digits, so that the panel did not know which sample was untreated or PEF treated. The panelists were asked to discuss the perceived quality characteristics and to detect the sample that they thought was closer to the fresh basil according to the smell and color and to describe the reason of their choice. It was decided that the panelists would taste only 3 samples of their choice, which during the pairwise comparison they perceived more similar to the fresh sample regarding smell and color. This decision can be explained by the fact that basil has a rather strong aromatic character that might affect the perception of the following tastes if too many samples are tried. Also, the

memory of the taste of all the samples tasted might faint if too many samples are tasted. The samples of the pesto and the dried basil were served directly on a plate, without presenting it on a piece of bread, and the panelists were asked to have a sip of water and take a bite of white bread after every sample tasted.

5.6 Statistical analysis

A curve fitting for both untreated and PEF treated samples was made in a statistics program, in an integrated development environment for R in the program version RStudio Desktop 0.99.896 (RStudio, Boston, USA). The curve fitting was made according to drying curves of an average of 3 sets of experimentally obtained data. The data was fit into two models, the Newton model and the Logarithmic model. The model with the better fit was selected through a comparison of the values for the coefficient of determination (R^2), the root mean square error (RMSE) and the sum of square error (SSE).

Statistical analysis of the results obtained by the experiments were made with RStudio Desktop 0.99.896 (RStudio, Boston, USA), where a one-way ANOVA was used to analyze significant differences between the different treatments and drying techniques (significant if p-vale < 0.05). In case there was a significant difference observed, a Tukey multiple comparison test was used to evaluate true differences in treatment means.

6 Results

6.1 Fluorescent Microscopy for the evaluation of the PEF treatment

6.1.1 Identification of electroporated guard cells by the PEF process

A few trials were needed to find the right conditions for the electrical treatment to achieve the electroporation of the guard cells, condition needed for keeping the stomata open irreversibly. In Figure 7 images of basil leaves treated with two different conditions of PEF are displayed. Unsuccessful PEF conditions (600V/cm, keeping the rest of the conditions the same as described in section 5.3) resulted in electroporated epidermal cells, but did not show any electroporation of the guard cells, as indicated by the white arrows in Figure 7 A. The conditions reported in section 5.3 resulted in electroporated guard cells (Figure 7 B). When observing both trials with lower magnification, no matter if the guard cells were electroporated (Figure 7 D) or not (Figure 7 C), uniform electroporation of the basil leaf tissue was obtained. Homogeneous staining of the nuclei in the electroporated cells indicated uniform electroporation (Figure 7 C and D). In the bottom of Figure 7 D, a dark region can be seen, which is an unfocused region of the camera.

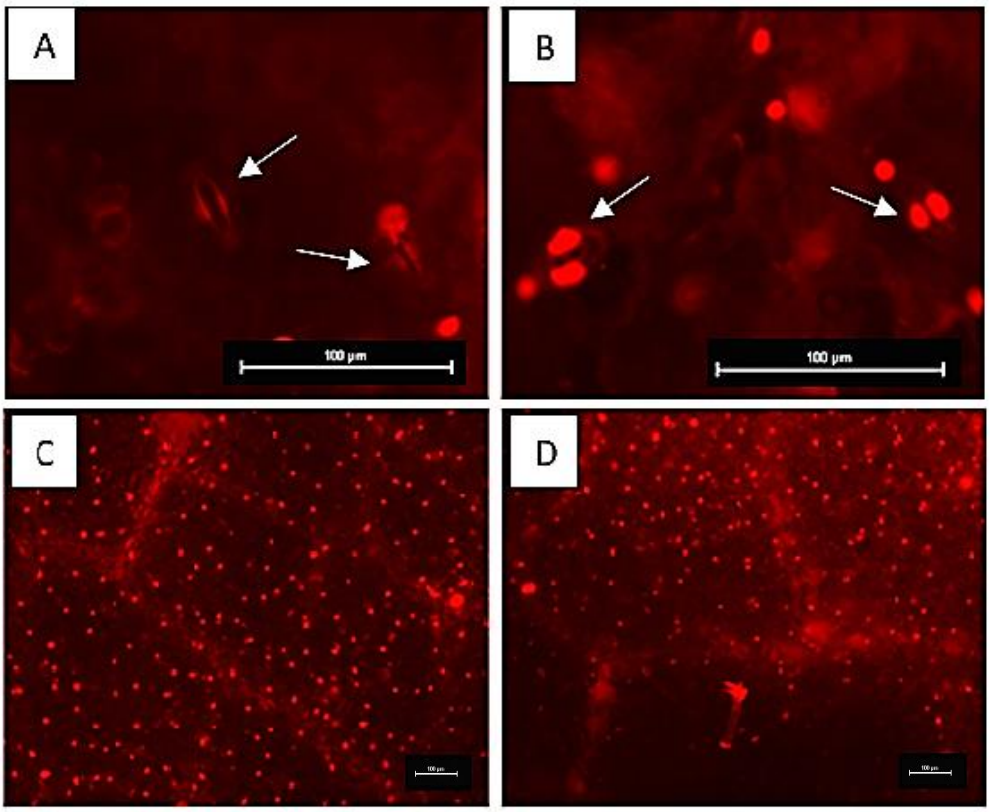

Figure 7 Fluorescent microscope images of PEF treated basil leaves in a 300 µM, conductive (130µs) PI solution. A) and C) display samples PEF treated with one train of 65 pulses with 600 V/cm, 150 µs pulse width, and 760 µs pulse space. B) and D) show samples PEF treated with one train of 65 pulses with 650 V/cm, 150 µs pulse width, and 760 µs pulse space. All scale bars indicate 100 µm.

6.1.2 Identification of cell viability of PEF treated leaves after storage

In Figure 8 the images of the cell viability test are displayed. Figure 8 A shows the positive control, 8 B the PEF treated sample and 8 C the negative control. The positive control and the PEF treated sample show a bright fluorescent green color induced by FDA, which indicates living cells. This shows that the PEF conditions induced a reversible permeabilization keeping the cells viable. The negative control shows clearly that the cells were killed with the PEF conditions used, as no green fluorescence was observed, indicating an irreversible electroporation of the basil leaf tissue. In addition, white circles indicate the essential oil glands normally keeping the aroma compounds of the leaves. In the negative control (Figure 8 C) all the essential oil cavities normally present embedded in the inferior epidermis were destroyed during the PEF treatment, as only black spots can be observed. Whereas in the positive control and the PEF treated sample (Figure 8 A and B), intact oil cavities, with the typical bulb shape can be seen.

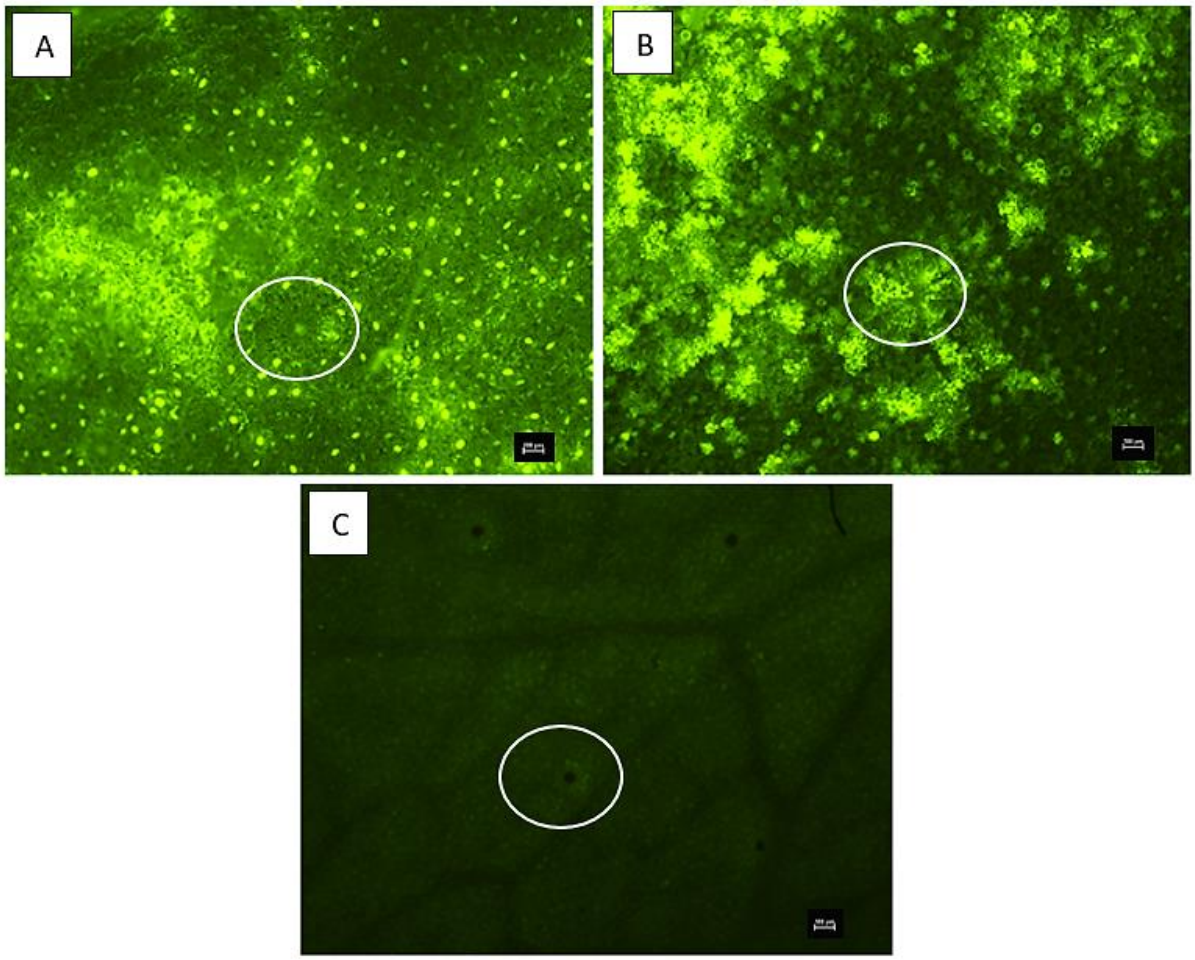

Figure 8 Fluorescent microscope images after incubation of basil leaves in 12 µm FDA solution. A) Positive control, fresh basil leaf, just detached from basil plant before FDA incubation. B) sample PEF treated with one train of 65 pulses with 650 V/cm, 150 µs pulse width, and 760 µs pulse space. C) Negative control, sample PEF treated with one train of 990 pulses with 650 v/cm, 400 µs pulse width and 800 µs pulse space. All scale bars indicate 100 µm. The white circles indicate the position of the essential oil glands of the basil leaf.

6.2 Drying processes

6.2.1 Air drying

Table 1 shows that both models give a rather good fit to the experimentally obtained drying curves, whereas the logarithmic model was found to be a better fit for both control and PEF treated basil leaves. This is explained by the higher coefficient of determination values (R^2), which give an idea about how well the fitted curve fits the actual data points. As an example, with an R^2 of 0.998, as obtained for the logarithmic model of the control sample, it can be said that 99.8 % of the experimentally obtained data points can be explained by the model. Additionally, the root mean square error (RMSE) and the sum of square error (SSE) are both lower for the logarithmic models than for the newton models. Both give an idea about the differences between the fitted values and the experimentally obtained values, the smaller they are, the smaller the deviation between the fitted and experimental values.

Table 1 Statistically obtained parameters by fitting the Newton model and the Logarithmic model onto experimentally obtained air drying data of untreated and PEF treated basil leaves.

Treatment and Model	**Equation**	**R^2**	**RMSE**	**SSE**
Control Newton Model	$MR = e^{(-0.00238*time)}$	0.9964	0.0449	2.332
Control Logarithmic Model	$MR = 0.853e^{(-0.00261*time)} + 0.0578$	0.998	0.0092	0.098
PEF Newton Model	$MR = e^{(-0.00616*time)}$	0.9922	0.0406	1.184
PEF Logarithmic Model	$MR = 0.799e^{(-0.00538*time)} + 0.0225$	0.9962	0.0110	0.087

Figure 9 is shows that the logarithmic model (B, red line) fits better onto the experimental data (blue line).

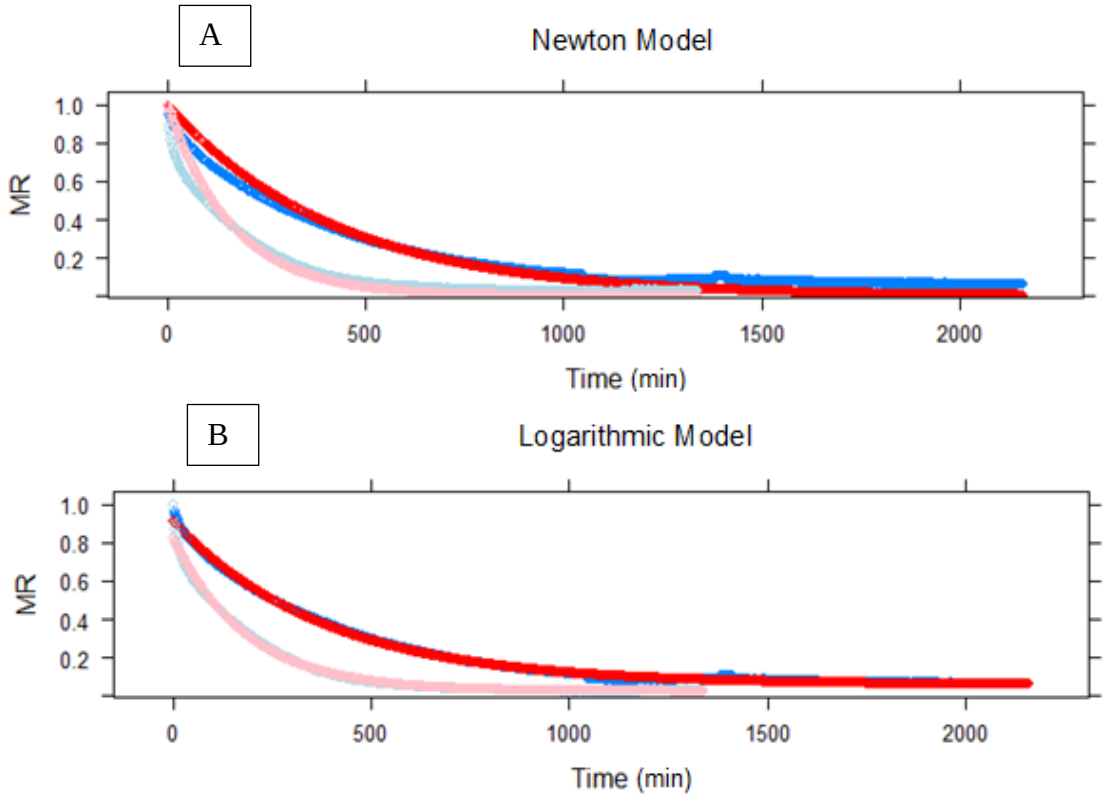

Figure 9 Model fitting of drying curves (moisture ration vs time plots), in R studio. A) Newton model fitted on to experimentally obtained convection air drying curves of untreated and PEF treated basil leaves at 40 °C. B) Logarithmic model fitted on to experimentally obtained convection air drying curves of untreated and PEF treated basil leaves at 40 °C.

It was decided to use the logarithmic models for both untreated and PEF treated samples to optimize the drying process and to calculate the drying time to obtain a moisture content of around 7.5 %, as shown in Table 2

Table 2 Experimentally obtained moisture content and water activity of untreated and PEF treated basil leaves at their calculated convective air drying time and effective moisture diffusivity to obtain a product with about 7.5 % moisture content with a drying temperature of 40°C.

Logarithmic model	**Effective Moisture Diffusivity (m^2min^{-1})**	**Calculated drying time "t" to obtain 7.5 % moisture content (h)**	**Experimentally obtained moisture content at time "t" (%)**	**Water activity at time "t"**
Control	$5.66*10^{-12}$	23	7.2 ± 1.0	0.48 ± 0.11
PEF treated	$1.25*10^{-11}$	10	7.9 ± 1.3	0.46 ± 0.14

Table 2 shows that the drying times calculated with the logarithmic model resulted in products with about 7.5 % moisture content. The difference in drying time between the untreated sample and the PEF treated sample was found to be 13 hours, which means that the control air dried sample needs 2.3 times longer to reach a moisture content of around 7.5 % than the PEF treated sample.

6.2.2 Vacuum Drying

The conducted trials to find the drying time for obtaining a product with a moisture content of around 7.5 % can be seen in Table 3. The drying times were found to be 15 hours for the untreated samples and 10 hours for the PEF treated basil samples, which is a drying time difference of 5 hours. This means that the vacuum drying time at room temperature for the untreated leaves is around 1.5 times longer than the vacuum drying time of the PEF treated sample.

Table 3 Experimentally obtained vacuum drying times at room temperature and their correspondent moisture contents of untreated and PEF treated basil leaves.

Control vacuum dried samples			PEF treated vacuum dried samples		
Drying time (h)	Moisture content (%)		Drying time (h)	Moisture content (%)	
35	4.6 ± 0.1		8	8.4 ± 0.7	
24	4.9 ± 0.2	Average	9	8.7 ± 1.0	Average
15	6.7 ± 1.3	7.3 ± 1.3	10	7.3 ± 1.0	7.0 ± 0.6
15	7.4 ± 0.3		10	7.0 ± 0.1	
15	7.9 ± 0.9		10	6.7 ± 0.4	
14	8.6 ± 0.9				

6.2.3 Freeze Drying

The freezing curves from the control and the PEF treated basil leaves obtained by experimental data showed that the freezing time to reach -30 °C was found to be at around 100 seconds (see Appendix, section 12.2). Additionally, it was seen that there was no difference between the freezing pattern of the untreated and the PEF treated basil leaves.

The conducted trials to find the drying time to obtain a moisture content of around 7.5 % can be seen in Table 4. The drying times were found to be 12 hours for the untreated basil leaves and 9 hours for the PEF treated basil leaves. This means that there was a difference of 3 hours between the untreated and the treated samples, which means that the freeze drying time for the untreated leaves is around 1.3 times longer than the PEF treated sample.

Table 4 Experimentally obtained freeze drying times and their correspondent moisture contents of untreated and PEF treated basil leaves.

Control freeze dried samples			PEF treated freeze dried samples		
Drying time (h)	Moisture content (%)		Drying time (h)	Moisture content (%)	
18	6.0 ± 0.1				
15	6.5 ± 0.4	Average	11	6.4 ± 1.2	Average
12	8.2 ± 0.5	7.6 ± 0.6	9	6.9 ± 0.7	7.6 ± 0.7
12	7.1 ± 0.3		9	8.0 ± 0.3	
12	7.5 ± 0.4		9	8.0 ± 0.4	
8	9.4 ± 0.8		7	8,9 ± 0.4	

6.3 Quality evaluation

6.3.1 Moisture Content and water activity

In Table 5 the moisture content of the different treatments and drying techniques are displayed with their corresponding water activity. There was no significant difference between the moisture content of the dried samples. This means that the results of all the following analysis could be compared between the different treatments and drying techniques without concern. There was no significant difference between the water activity obtained by the different drying methods, with exception of the water activity of the control air dried sample compared with the PEF vacuum dried sample. All dried samples are safe to consume as microorganisms do not grow below a water activity of 0.6 (AquaLab, 2016).

Table 5 Drying times, moisture content and water activity of basil leaves dried with different techniques. Leaves were either untreated or pre-treated with PEF. The final moisture content of the leaves was about 7.5 %. Different letters within a column indicate statistical significance ($p < 0.05$)..

Treatment and drying technique	**Drying time (h)**	**Moisture content (%)**	**Water activity**
Fresh basil	/	92.5 ± 0.3 a	0.96 ± 0.00 b
Control air dried	23	7.2 ± 1.0 b	0.48 ± 0.11 ac
PEF air dried	10	7.9 ± 1.3 b	0.46 ± 0.14 a
Control vacuum dried	15	7.3 ± 1.3 b	0.32 ± 0.03 a
PEF vacuum dried	10	7.0 ± 0.6 b	0.28 ± 0.03 ad
Control freeze dried	12	7.6 ± 0.6 b	0.30 ± 0.03 a
PEF freeze dried	9	7.6 ± 0.7 b	0.41 ± 0.04 a

6.3.2 SEM examination

Examination of fresh basil leaves

The ESEM pictures of the fresh basil leaf surface structure could not be used for comparison, as the right conditions for the analysis could not be found. The samples dried during the examination time in the microscope and resulted in pictures with collapsed cell structure. Focus on the samples could not be achieved with higher chamber humidity during the time of experimentation in this master thesis.

Structure examination of dried basil leaves

Figure 10 shows all of the dried leaves shrunk in size during the drying process. To visualize this better, fresh leaves in the size range used for the drying were placed between the dried products for comparison. Further it can be observed that the freeze dried leaves shrunk the least, followed by the vacuum dried leaves, but for both the techniques no clear difference between the untreated and the PEF treated leaves can be observed with the naked eye. The convective air dried leaves show the largest reduction in size, where it can be clearly observed that the control air dried leaves shrunk more comparing them to the PEF air dried samples.

Figure 10 Shrinkage of untreated and PEF treated basil leaves dried with different techniques in relation to their fresh standard.

SEM examination of cell collapse during drying process

The SEM pictures displayed in Figure 11 give a better understanding about the surface structure of the tissue after drying. It can be clearly seen that control air dried leaves undergo severe collapse, whereas the collapse in the PEF treated sample is diminished, as clearer cell structures can be seen. For the vacuum dried basil leaves it is the other way around, the PEF treated sample shows more cell collapse when comparing it to the untreated control. In comparison to the air dried leaves can be said that the control air dried sample displays the most collapse, followed by the PEF vacuum dried sample, then the PEF air dried sample and finally the control vacuum dried sample. When looking at the structure of the freeze dried leaves, both control and PEF treated, it can be seen that no clear collapse of the cells can be observed, the surface structure of the cells seems to be intact. When comparing the surface structure of the freeze dried samples to the rest of the samples it can be seen that the freeze dried samples show the least amount of cell collapse and so preserved the original cell shape the best.

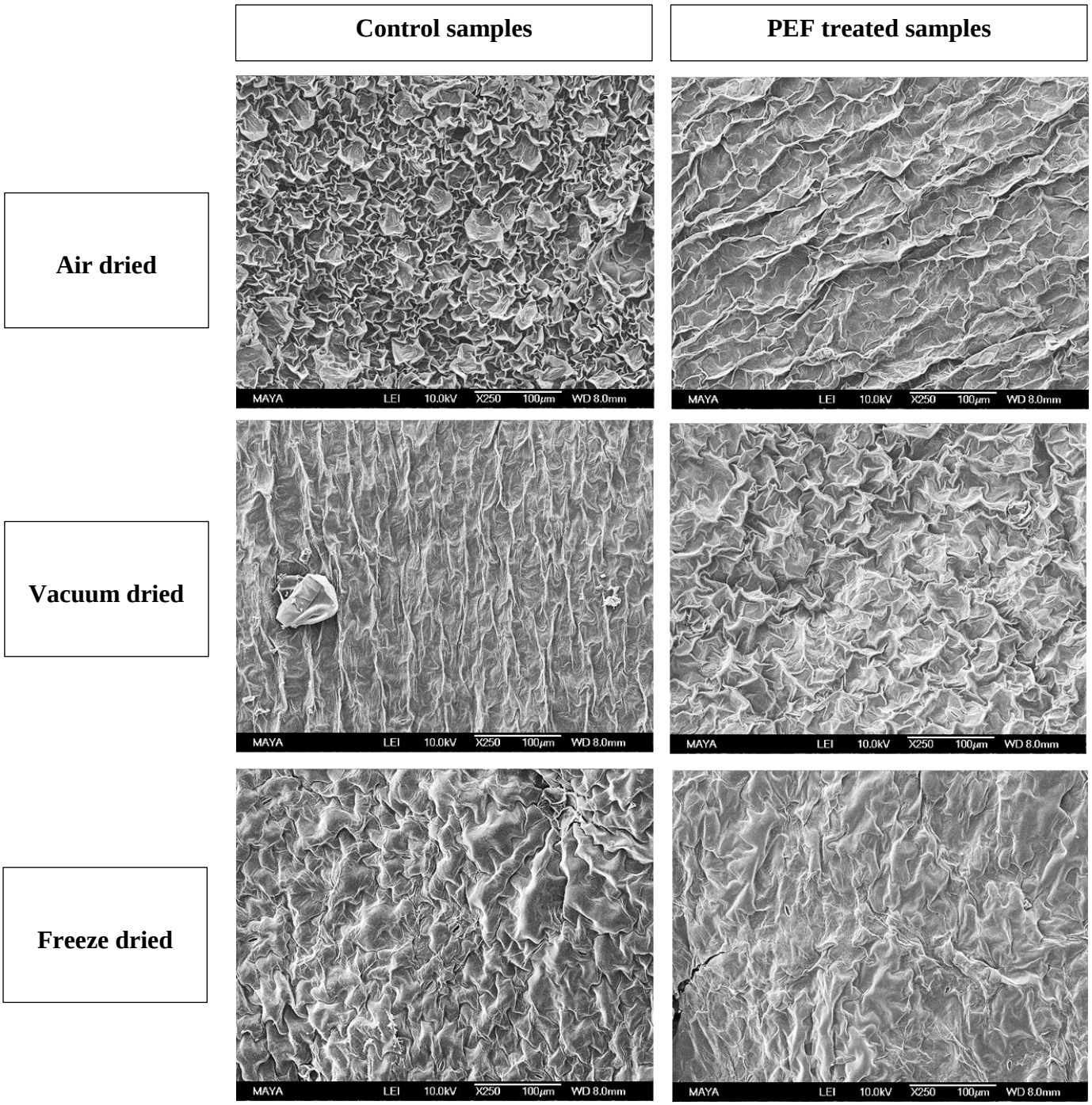

Figure 11 Scanning electron microscope pictures of the top surface of untreated and PEF treated basil leaves dried with different techniques. Pictures were taken at a working distance of 8 mm, with an acceleration voltage of 10 kV at a magnification of 250. The scale bars indicate a span of 100 µm.

SEM examination of stomata aperture during the drying process

When observing the structure of the samples at higher magnification, it was possible to see the difference in stomata opening (Figure 12). It can be observed that the stomata in the control air dried and the control vacuum dried samples closed during the drying process, whereas in all the other samples the stomata stayed open throughout the entire drying time.

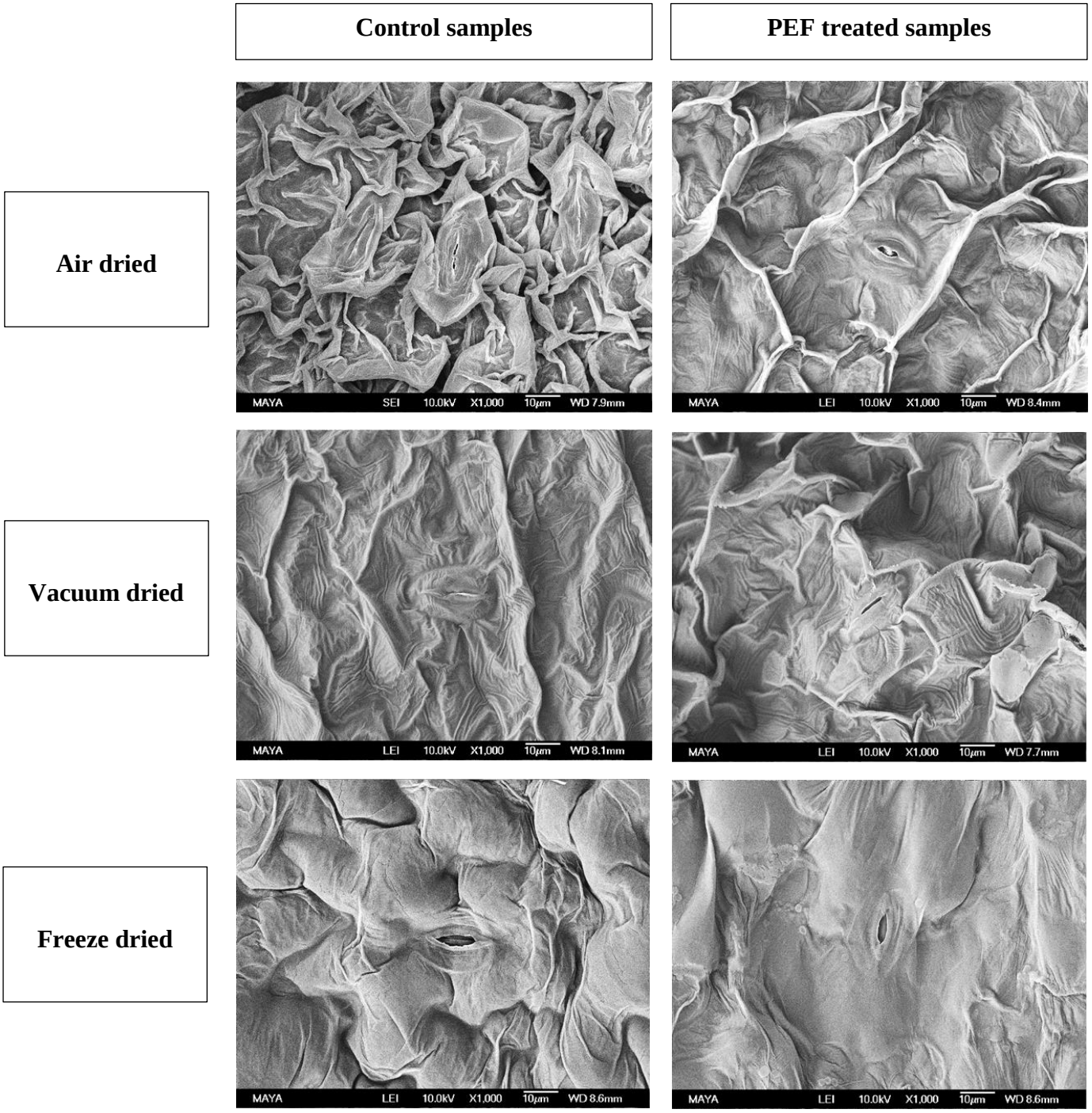

Figure 12 Scanning electron microscope pictures of the top surface of untreated and PEF treated basil leaves dried with different techniques. Pictures were taken at a working distance between 7.7 and 8.6 mm, with an acceleration voltage of 10 kV at a magnification of 1000. The scale bars indicate a span of 10 µm.

SEM examination of glandular trychomes preservation during the drying process

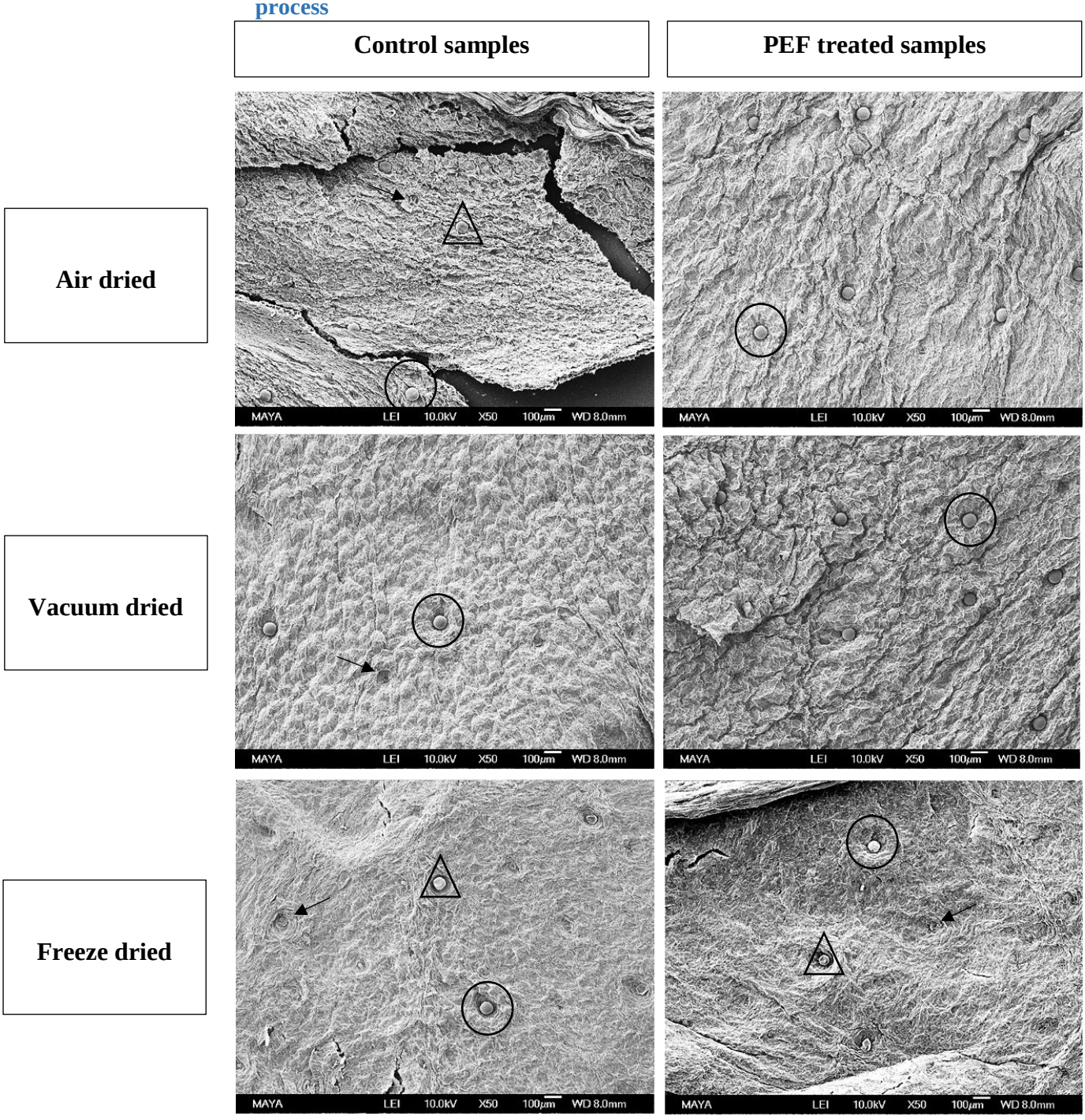

Figure 13 Scanning electron microscope pictures of the bottom surface of untreated and PEF treated basil leaves dried with different techniques. Pictures were taken at a working distance of 8 mm, with an acceleration voltage of 10 kV at a magnification of 50. The scale bars indicate a span of 100 µm. Circles are indicating examples of intact oil cavities, triangles are indicating examples deformed oil cavities and arrows are indicating examples of destroyed oil cavities.

Not only the cell structure was observed under the SEM, also the essential oil glands were examined (Figure 13). For the PEF air dried and the PEF vacuum dried sample the essential oil glands stayed well embedded in the inferior epidermis and kept their round shape indicating their preservation (examples are visualized with a circle in Figure 13). By looking at the images of all the control samples and the PEF freeze dried sample in Figure 13, it can be observed that only a few single essential oil glands kept their round shape (example

visualized by a circles), and so stayed intact. In these pictures, most of the essential oil cavities are either deformed (example visualized by a triangle) or fully destroyed (example visualized with an arrow), where only the notch in which the essential oil glands were embedded remained.

6.3.3 Rehydration

Rehydration capacity at 100°C

The rehydration curve at 100°C is shown in Figure 14. It was observed that after the initial rapid rehydration of the dried basil leaves in boiling water the weight increase over time is rather small. So, the focus in this curve was to examine the rehydration capacity during the first few minutes. A fast rehydration of all the samples in the first few minutes was observed, where no significant difference between the samples was obtained. This can be related to the rather high variability in rehydration capacity of the triplicates of every sample, as seen by the error bars.

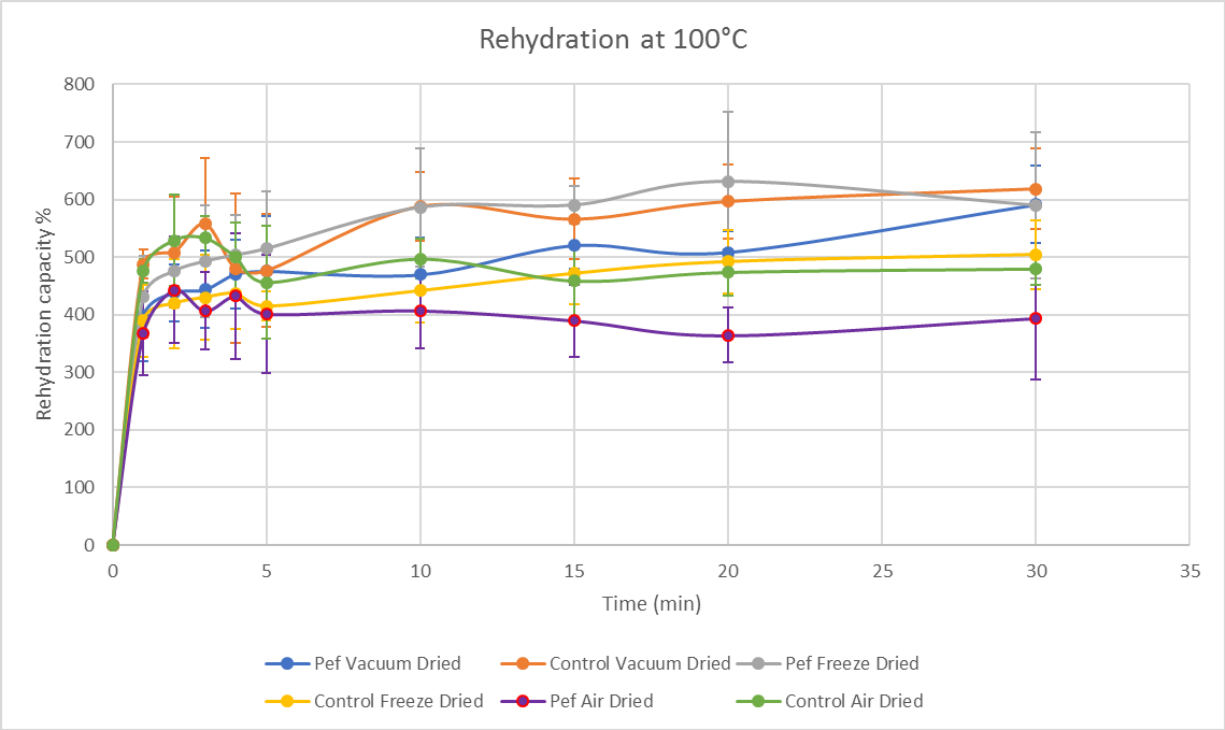

Figure 14 Rehydration curve of untreated and PEF treated basil leaves dried with different techniques at 100°C, with standard deviation error bars.

Another aspect that was assessed during the rehydration at 100°C was the ability of the dried basil leaves to float on top of the boiling water, as floatability is a quality parameters of dried herbs in instant soups (Feyecon, personal communication). It was observed that all the basil leaves, independently on the treatment or drying technique, floated on the top layer of the water throughout the entire rehydration test.

Rehydration capacity at room temperature

Figure 15 displays that constant weight for all the samples was achieved after around 24h.

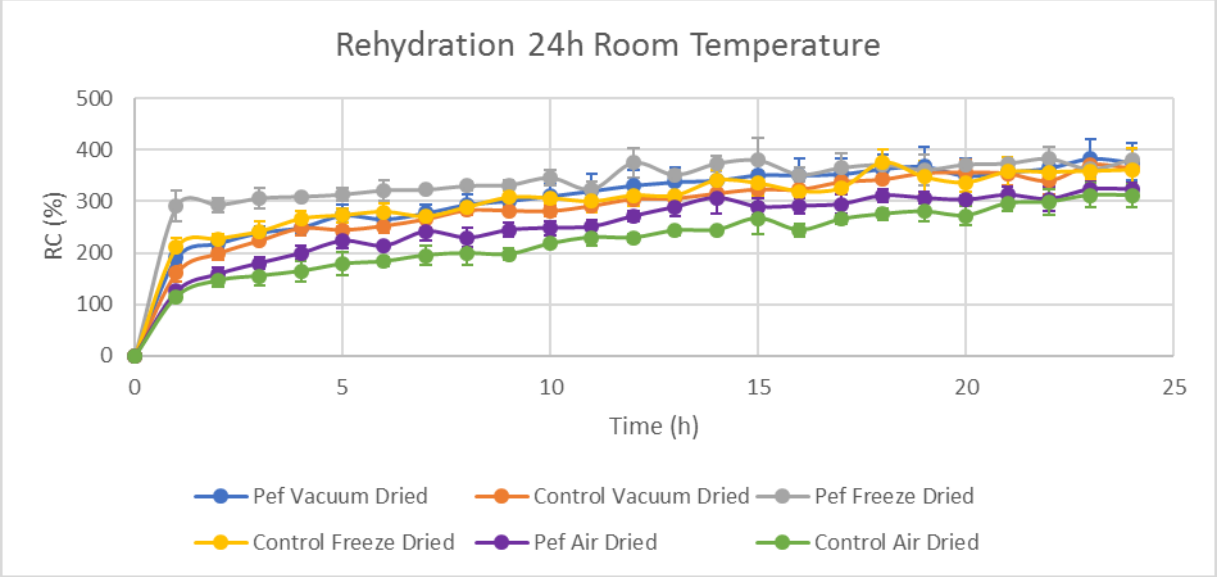

Figure 15 Rehydration curve of untreated and PEF treated basil leaves dried with different techniques at room temperature, with standard deviation error bars.

Within the first hour all the samples show a high increase in rehydration capacity, whereas the increase over the following hours is rather small compared to the first hour. This pattern is more dominant in freeze dried samples.

The maximum rehydration capacity of all the basil leaves after 24 hours was assessed with ANOVA, where no significant difference between untreated and PEF treated samples dried with different techniques was observed.

In addition, the percentage of weight recovered by the different leaves in comparison to the weight of a fresh leaf was evaluated (Figure 16). There was no significant difference between the different samples obtained.

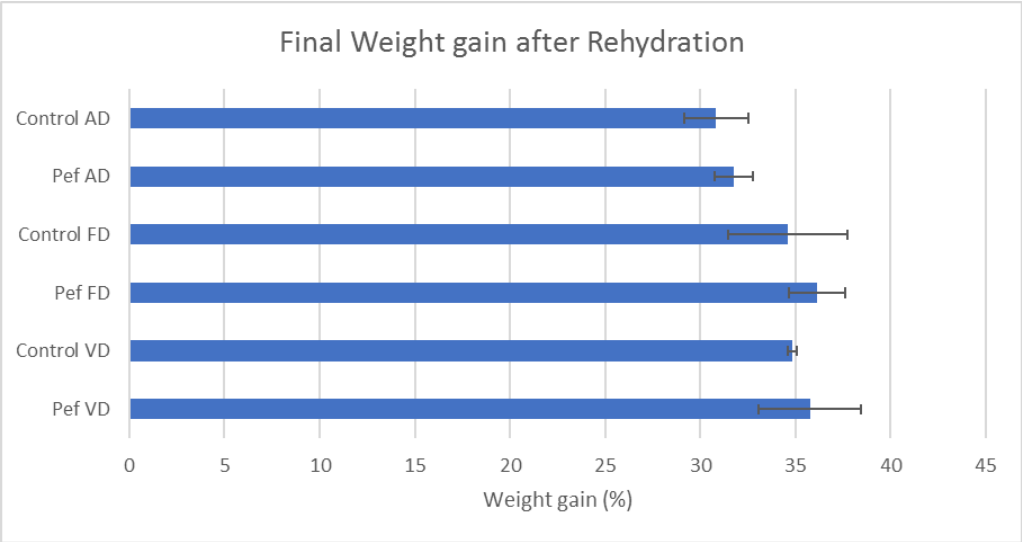

Figure 16 Percentage of weight gain after rehydration (as percentage of the weight of the fresh leaf) by untreated and PEF treated basil leaves dried with different techniques. Error bars display the standard deviation.

Another interesting parameter to examine is the speed of rehydration. For the air dried samples, Figure 17 A shows that the PEF treated samples takes less time to reach the maximum rehydration capacity than the untreated sample. The PEF treated sample reaches its maximum rehydration capacity after 14 hours in contrast to the control samples which takes 21 hours. Figure 17 B shows that the PEF freeze dried sample reaches its maximum rehydration capacity already after 12 hours, whereas the control freeze dried sample needs around 18 hours. For the vacuum dried samples, as observed in Figure 17 C, both untreated and PEF treated samples reach their maximum rehydration capacity after 19 hours.

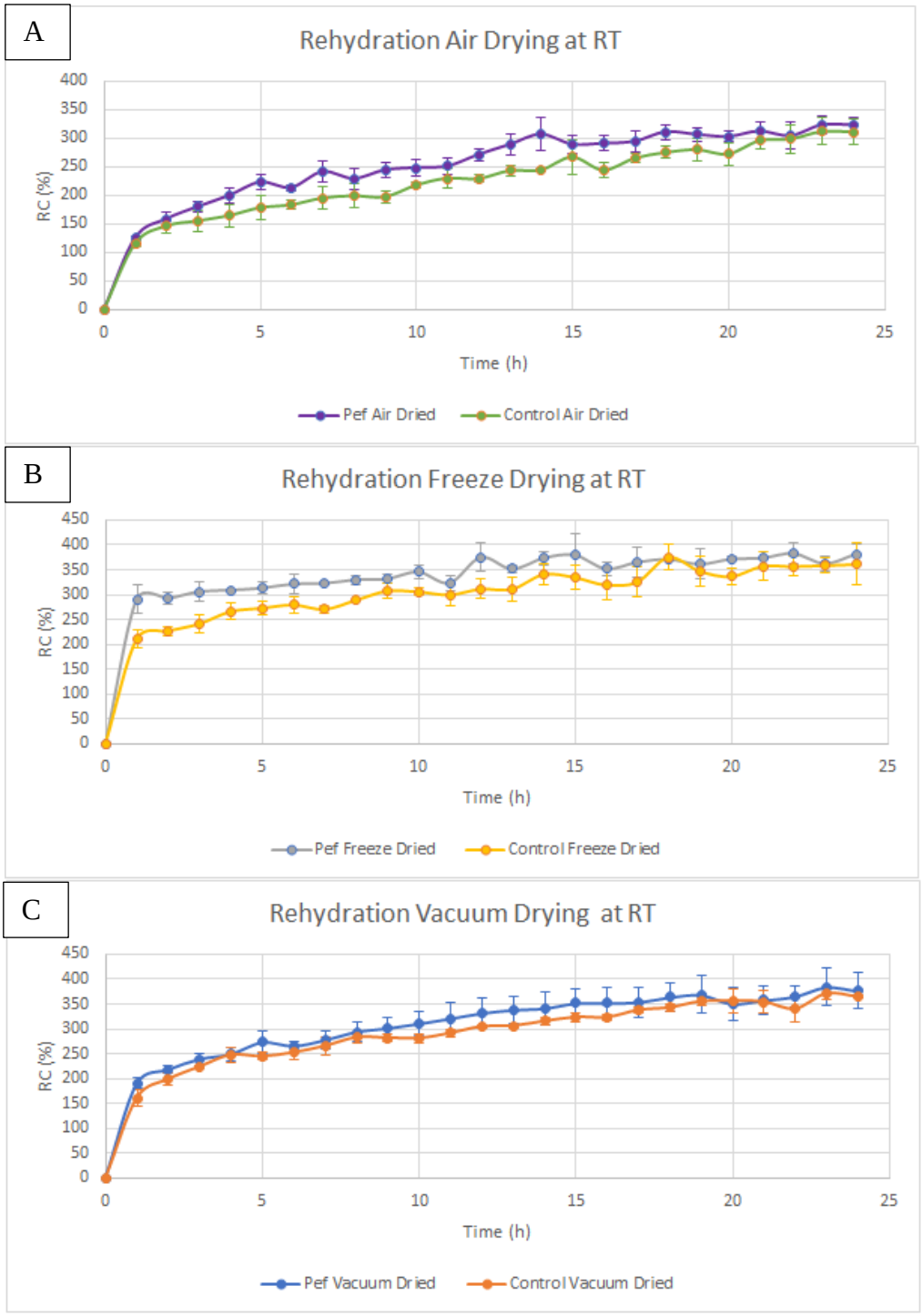

Figure 17 Rehydration curve of dried basil at room temperature. A) displays the rehydration curve of untreated and PEF treated air dried basil leaves. B) displays the rehydration curve of untreated and PEF treated freeze dried basil leaves. C) displays the rehydration curve of untreated and PEF treated vacuum dried basil leaves. Error bars indicate standard deviation.

6.3.4 Color Measurement

The color differences induced by the drying processes as perceived by the human eye, are displayed in Figure 18, where fresh leaves were placed between the dried samples for better comparison. It can be seen, that all samples darkened during the drying process, except the PEF

freeze dried sample, which shows bright, greyish particles within the sample indicating a color loss. The control freeze dried sample changed into a dark green color. Both air dried samples changed into an olive green color, whereas the vacuum dried samples show the least color change after drying. When comparing the commercial sample to the other samples it can be described as brown- yellowish rather than green.

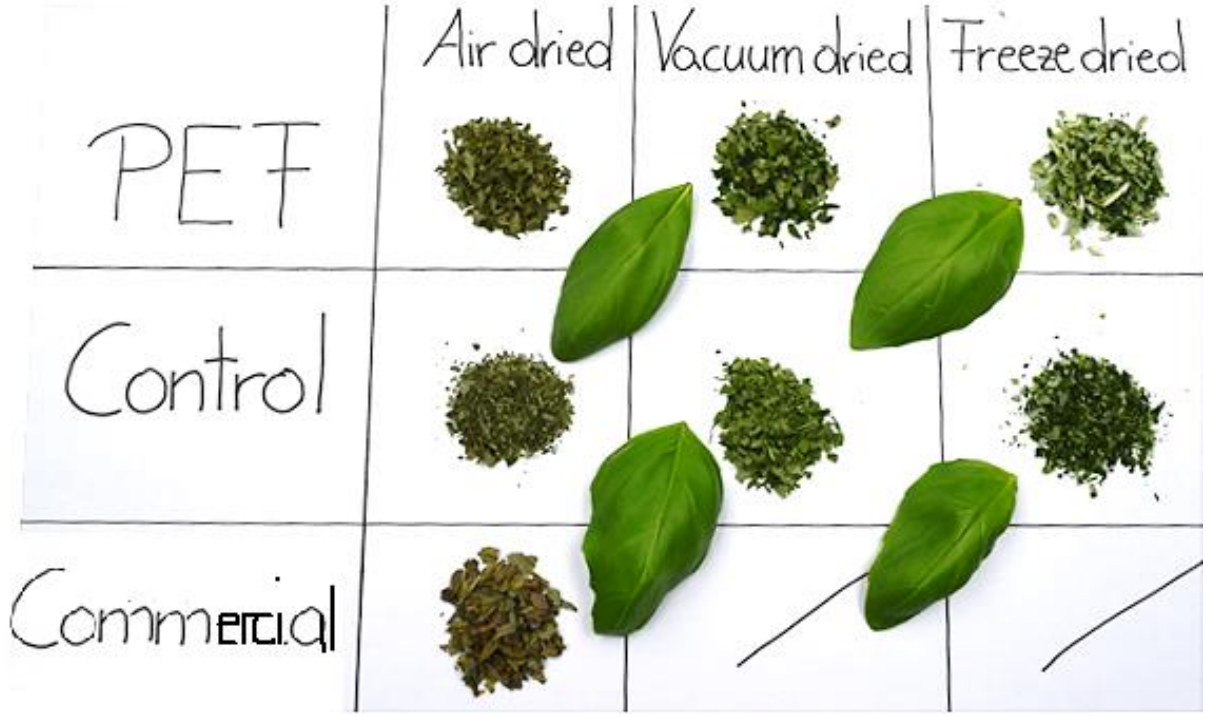

Figure 18 Color difference of fresh basil leaves and untreated and PEF treated basil leaves dried with different techniques and a commercial dried basil sample.

CIE *L*a*b** analysis

The CIE *L*a*b** coordinates for the fresh leaves and the dried sample are displayed in Table 6. According to the L* values, it was observed that the fresh leaves are significantly brighter than the dried products, with the exception, of the lightness between the fresh leaves and the PEF treated freeze dried leaves. This means that all the samples except the PEF treated freeze dried leaves got darker during the drying process. The darkening of the untreated samples independently on the drying technique, was not significantly different from each other. Additionally, there was no significant difference in lightness between the PEF treated vacuum dried sample and the air- and freeze dried control samples. Whereas the PEF air dried sample was significantly darker than all the other samples after drying.

*Table 6 CIE L*a*b* coordinates and the calculated total color change for untreated and PEF treated basil leaves compared to fresh basil leaves. Different letters within a column indicate statistical significance ($p < 0.05$)*

Treatment and drying technique	**L***	**a***	**b***	**ΔE***
Fresh basil	43.53 ± 2.1 a	-13.79 ± 1.4 a	44.95 ± 2.5 a	/
Control air dried	40.42 ± 0.5 b	-4.25 ± 0.2 c	15.63 ± 0.1 b	30.99 ± 0.1 a
PEF air dried	36.29 ± 0.2 c	-3.99 ± 0.1 c	16.63 ± 0.3 bd	30.83 ± 0.4 a

Control vacuum dried	41.04 ± 0.3 bd	-6.84 ± 0.0 b	17.44 ± 0.1 bd	28.48 ± 0.1 b
PEF vacuum dried	39.25 ± 0.5 be	-6.14 ± 0.0 bd	16.84 ± 0.1 bd	29.44 ± 0.1 c
Control freeze dried	40.45 ± 0.5 b	-6.52 ± 0.0 b	15.01 ± 0.3 be	30.96 ± 0.2 a
PEF freeze dried	44.53 ± 1.2 a	-6.16 ± 0.3 be	13.58 ± 0.6 c	32.32 ± 0.7 d

When looking at the values for greenness (a*values) in Table 6, the color of the fresh basil leaves is indicating a color which is significantly closer to green than the color of the dried samples. It was detected that the greenness of untreated and PEF treated samples dried with the same technique were not significantly different from each other. But the greenness when comparing samples dried with different techniques showed significances with some exceptions. The greenness of the control freeze dried sample was not significantly different from the greenness of both untreated and PEF treated vacuum dried samples. Also, the PEF freeze dried sample did not show any significant difference in greenness from the control vacuum dried sample. It can be said that the air dried samples show a greenness which is furthest away from the green color of the fresh sample.

The b*values in Table 6 show that the fresh basil leaves are displaying a color which is significantly more migrating towards the color yellow than the dried products. When comparing the yellowness of the dried products with each other it was detected that there was no significant difference in yellowness between control and PEF treated samples for both air- and vacuum drying. Where the PEF treated samples of both techniques are also not significantly different from each other and the control of the respectively other technique. Additionally, there was no difference in yellowness between the control air dried and the control freeze dried sample detected. These measurements lead to the conclusion that the yellowness of the PEF freeze dried sample is the one significantly most different from the yellowness of the fresh sample.

When looking at the calculated total color change reported in Table 6, it can be seen that the PEF freeze dried sample show a significant higher color change than the other samples and the least color change was observed in the vacuum dried samples. As the difference in ΔE of about 2.3 corresponds to a just noticeable difference it can be seen that all of the products went through rather drastic color changes. Almost all of the color changes are significantly different from each other, expect the color change of the control air dried sample compared with the change of the PEF treated sample dried with the same technique. Additionally, both air dried samples do not show any significant difference in color change when compared to the control freeze dried sample.

CIE *L*C*h* analysis

The CIE *L*C*h* coordinates for the fresh leaves and the dried sample are displayed in Table 7, where the results obtained by the L* values were already stated in Table 6, but they were still added for the sake of completeness. It can be seen that all the hue angles are in the range of green color, where every angle is significantly different from the other and perceived by the eye as different, because 1° difference in hue makes already a slight difference in color perception. Both air dried samples show the closest hue compared to the fresh sample and the PEF freeze dried sample indicates a greenness furthest away from the fresh leaves.

*Table 7 CIE L*C*h coordinates and the calculated change in h and chroma of untreated and PEF treated basil leaves compared to fresh basil leaves. Different letters within a column indicate statistical significance ($p < 0.05$)*

Treatment and drying technique	**L***	**h (°)**	**C* (°)**	**Δh (°)**	**ΔC (°)**
Fresh basil	43.5 ± 2.1 a	107.0 ± 1.0 a	47.0 ± 2.7 a	/	/
Control air dried	40.4 ± 0.5 b	105.2 ± 0.1 b	16.2 ± 0.1 b	-1.8 ± 0.1 a	-30.8 ± 0.1 a
PEF air dried	36.3 ± 0.2 c	103.5 ± 0.1 c	17.1 ± 0.3 bcd	-3.5 ± 0.1 b	-29.9 ± 0.3 b
Control vacuum dried	41.0 ± 0.3 bd	111.4 ± 0.1 d	18.7 ± 0.1 c	4.4 ± 0.1 c	-28.3 ± 0.1 c
PEF vacuum dried	39.3 ± 0.5 be	110.0 ± 0.0 e	17.9 ± 0.1 c	3.0 ± 0.0 d	-29.1 ± 0.1 d
Control freeze dried	40.5 ± 0.5 b	113.5 ± 0.3 f	16.4 ± 0.3 b	6.4 ± 0.3 e	-30.7 ± 0.3 a
PEF freeze dried	44.5 ± 1.2 a	114.4 ± 0.1 g	14.9 ± 0.7 be	7.4 ± 0.2 f	-32.1 ± 0.7 e

When looking at the values for chroma displayed in Table 7, it can be seen that the saturation of the green color (C*) of the fresh leaves is significantly higher than for the dried samples. When looking at the dried samples, not all values of chroma are significantly different from each other. This does not mean that the samples will be perceived similarly, as the chroma indicates only the saturation related to the color shown by the hue angle, which was different for every sample.

The change in hue and the change in chroma, reported in Table 7 give an idea about the difference in color and its saturation of the sample after drying compared to the fresh basil leaves. It was observed that the control air dried sample showed the lowest change in chroma, whereas it changed towards to a yellow color, such as the PEF air dried sample as well, which can be seen by the negative hue angle change. Vacuum- and freeze dried samples have faced bigger color changes, to a greener color, shown by the positive hue angle change in Table 7, which indicates a shift of the color towards the green color on the hue angle wheel. The freeze dried samples underwent the biggest changes in hue angle and saturation after drying, in particularly the PEF treated freeze dried sample. The change in saturation is rather big for all the dried products, where a loss in saturation can be observed (Table 7), resulting in a less intense color. The sample with the least change in saturation was the control vacuum dried sample.

6.3.5 Sensory Evaluation

Dried basil focus group discussion

Initial consumer information

Initially general information about the use and the most important quality aspects of dried basil was obtained by the panelists. Where 85.8 % stated that they were using herbs frequently when cooking, only one panelist does not cook with herbs. Among the panelists, 71.5 % commonly use basil when cooking and 57.2 % cook using dried basil.

The panel discussed the use of basil during their cooking sessions, where the dishes in which they use basil were the following:

- Fresh basil: dressing, pesto, ice cream, pizza and pasta, salad,
- Dried basil: soups, tomato-sauces, meat.

When it comes to what characteristics of the product the panelists look at when purchasing dried basil, 100 % chose the color, and it was specified that the product should not have a brown color. When asking for the most appealing color of dried basil, all panelist stated that a dark green color would be preferred. Other characteristic that the panelists look at when purchasing dried herbs are price, the place of origin and the brand.

Standard description

The panelists were asked to describe the color, smell and taste of fresh basil and a commercial sample of dried basil. Their comments are reported in Table 8.

Table 8 Description of sensorial properties of fresh basil and a commercial dried basil sample

Sample	Color and appearance	Smell	Taste
Fresh	Fresh green, bright, shiny.	Fresh, aromatic, basil aroma, grassy sensation.	Bitter, a little sour, a spicy/minty aftertaste, fresh, weak flavor.
Commercial dried basil	Dark, dull, brown, greyish, olive green with hints of yellow, looks like a mixed spice product.	Herb mixture, not like basil, salty, one panelist stated it smells like a medicine and hospital.	Not like basil, and not fresh. It was associated to seaweed, tea and the bark of a tree.

Pairwise comparison of color and smell of dried basil samples

The first pair of dried basil samples to be assessed was the control and the PEF treated freeze dried samples. The panellists described the color and the smell of the samples as reported in Table 9. They decided which of the two samples they think is closest to the fresh sample and accordingly will be tried later during the focus group discussion. Table 9 shows that the control freeze dried sample was defined by the majority as being the closest one to the fresh sample according to its color and appearance. Among the panellists 71.5 % wanted to taste the PEF treated freeze dried sample based on its smell. Only two panellists defined the control freeze dried sample to be closer in smell to the fresh basil leaves, which were the panellists that are commonly cooking with dried basil.

Table 9 Comparison of sensorial properties of untreated and PEF treated freeze dried basil leaves to fresh basil as standard

Sample	Color and appearance	Smell
PEF freeze dried	1 panelist stated: color of this sample closer to the fresh. (2 panelists could not see difference). Greyish, darker green.	71.5 % of the panelists stated: smell of this sample closer to the fresh sample. Basil smell, keeps aroma in the air longer.

Control freeze dried	57.2 % of the panelists stated: color of this sample closer to the fresh basil. Looks fresher, more shiny color, more light green.	28.5 % of panelists stated: smell of this sample closer to the fresh sample. Intense smell, fresh.

The comparison and description of the color and smell of the untreated and PEF treated air dried samples is reported in Table 10. All the panelists stated that the PEF air dried samples were more appealing than the control air dried samples. The color of the PEF air dried sample was found to be closer to the fresh basil by 85.8 % of the panelists. When it comes to the smell 71.5 % of the panelists found the PEF air dried sample to be closer to the fresh basil. Only two panelists, both not commonly cooking with dried basil chose to taste the control air dried sample, where one of them described the color of this sample to be closer to the fresh basil leaves.

Table 10 Comparison of sensorial properties of untreated and PEF treated air dried basil leaves to fresh basil as standard

Sample	**Color and appearance**	**Smell**
PEF air dried	85.8 % of the panelists stated: color of this sample closer to the fresh basil. Green.	71.5 % of the panelists stated: smell of this sample closer to the fresh sample. Stronger basil smell, more intense, smells like fresh.
Control air dried	1 panelist stated: color of this sample closer to the fresh; Greyish, dull, yellowish, looks more brittle.	28.5 % of panelists stated: smell of this sample closer to the fresh sample. Herb mix, like any leaf.

The comparison and description of the last pair of samples, which were the untreated and PEF treated vacuum dried samples, is reported in Table 11. Only one panelist found the control vacuum dried sample to be closer to the fresh basil leaves in terms of color. Whereas 100 % of the panelists perceived the smell of the PEF vacuum dried sample as the one closer in smell to the fresh basil leaves, which means that all panelists tasted the PEF vacuum dried sample in the next stage of the sensorial evaluation process.

Table 11 Comparison of sensorial properties of untreated and PEF treated vacuum dried basil leaves to fresh basil as standard

Sample	Color and appearance	Smell
PEF vacuum dried	85.8 % of the panelists stated: color of this sample had closer to the fresh basil. More yellowish-green, brighter, shiny color.	100 % of the panelists stated: smell of this sample closer to the fresh sample. More intense basil smell, more fresh, mild in its flavor.
Control vacuum dried	1 panelist stated: color of this sample closer to the fresh; Greyish, more dull, more yellowish.	Smelled different than basil.

It was observed that the PEF treated samples resulted to be the samples closer in smell to the fresh samples, when compared to the untreated sample dried with the same technique. Additionally, for the air dried and the vacuum dried samples, the PEF treated leaves were perceived to be closer in color to the fresh basil than their control samples. This was not the case for the color of the freeze dried samples, here the color of the control sample seem to be closer to the color of the fresh basil leaves.

Examination of taste attributes of three chosen samples

The description of the chosen samples founder reported in Table 12. The PEF treated vacuum dried sample was described by the panelists as the closest to the fresh basil leaves when it comes to taste. The panelists were asked to rank the comparison of the taste of the 3 samples examined. Among the panelists, 85.8 % of the panelists stated that the taste of the PEF treated vacuum dried sample was the closest to the fresh sample, followed by the freeze dried samples, independently on the treatment of the sample being tasted. One panelist stated that the PEF freeze dried sample was closer in taste to the fresh basil than the PEF vacuum dried sample. 100 % of the panelists ranked the air dried samples, independently on the treatment, as the furthest away when comparing the taste to the fresh sample.

Table 12 Comparison and description of taste of untreated and PEF treated samples dried with different techniques to fresh basil as a standard

Sample	Taste
PEF freeze dried	Basil taste, not very intense flavor, sour aftertaste, smooth texture, taste remains for long time.
Control freeze dried	Light basil taste, basil flavor almost not detectable in aftertaste, flavor has sharpness, unpleasant aftertaste detected, dull aftertaste, sticky.
PEF air dried	Far from fresh sample, weak basil flavor, lack in flavor, bitter, not fresh, dusty, a bit grassy, not smooth texture, rough.
Control air dried	Basil flavor detected, not strong basil taste, not as fresh, hard mouth feel.
PEF vacuum dried	Close to fresh, mouth feel close to fresh, smooth, sourness like in fresh one, intense basil flavor, a bit salty.

Pesto focus group discussion

Initial consumer information

Initially, general information about the use of pesto was obtained, where 50 % of the panelists stated that they commonly consume pesto and the other 50 % do not normally eat it. Out of the 50 % of the panelists who consume pesto, one person consumes it every week and the rest 1-2 times a month. The main way of consumption is with pasta.

When it comes to the characteristics of the product the panelists look at when purchasing pesto, 100 % stated the color, where it was specified that the product should have a dark green color. Other characteristics stated were brand and an eco label.

Standard description

The panelists were asked to describe the color, smell and taste of pesto made with fresh leaves and a pesto made with commercial dried basil. Comments from the panelists are reported in Table 13.

Table 13 Description of sensorial properties of pesto made of fresh basil and a pesto made of commercial dried basil

Sample	Color and appearance	Smell	Taste
Pesto from fresh leaves	Bright color, intense green, yellowish. No separation of layers and ingredients, heavy texture, homogenized.	Pesto, parmesan cheese, weak smell of basil, fresh, leafy, nutty, oily.	Fresh basil (not only other ingredients), fresh, smooth mouth feel, not bitter, a bit grassy.
Pesto from commercial dried basil	Less green color, not appealing, many different colors of basil (black, brown, grey) Separation of layers (oil layer on top), flakey, ingredients floating around, like tea.	No basil, or very weak basil smell cheesy, less leafy, nutty, no freshness.	Taste of cheese comes through, basil is weak, basil almost not detected, dry texture (unpleasant), salty, bitter.

Pairwise comparison of color and smell of dried basil samples

The color and appearance of the pesto samples analysed are shown in Figure 19.

Figure 19 Pesto samples assessed during focus group discussion. Pesto samples from left to the right: fresh, PEF vacuum dried (153), Control vacuum dried (629), PEF freeze dried (732), Control freeze dried (509), PEF air dried (410), Control air dried (827), commercial dry product.

The first pair of pesto assessed consisted of the control and PEF treated vacuum dried basil samples. The panellists described the color and the smell of the samples as shwon in Table 14. They decided which of the two samples they think is closest to the fresh sample and chose the closest sample for a later evaluation. The color of both samples was described to be a lot darker than then the color of the fresh pesto. The vacuum dried sample being closer to the fresh samples according to appearance and color was found to be the PEF treated sample by 83.5 % of the panellists. Even though they found the appearance of the PEF treated sample to be closer to the fresh pesto, the same percentage of panellists detected the control samples to be the closest to the fresh standard when it comes to smell. It was interesting to see, that all the panellists decided to taste the sample which provides the attributes of smell closer to the fresh sample, out ruling the appearance attributes.

Table 14 Comparison of sensorial properties of pesto made of untreated and PEF treated vacuum dried basil leaves to pesto made of fresh basil leaves

Sample	Color and appearance	Smell
PEF vacuum dried pesto	83.5 % of the panelists stated: color/look of this sample closer to the fresh basil. Brighter, clearer, oil has taken on more green color, more liquid, less oil on top.	1 panelist stated: smell of this sample closer to the fresh sample. Slight basil smell, oily, cheesy.
Control vacuum dried pesto	1 panelist stated: color/look of this sample closer to the fresh. Yellowish, turbidity, small oil layer on top, bigger separation, looks pasty.	83.5 % of the panelists stated: smell of this sample closer to the fresh sample. More basil smell, cheesy.

The second pair of samples analyzed was the pesto prepared from the untreated and PEF treated freeze dried samples. Comments regarding comparison and description are shown in Table 15. The color of both samples was described to be a lot darker than then the color of the fresh pesto. In Table 15 can be seen that 50.1 % of the panelists found the PEF freeze dried sample to be the closer one to the fresh standard according to appearance. Additionally, 100 % of the

panelists chose the PEF treated freeze dried sample as the more similar in smell to the fresh basil pesto. It seems like the detected basil smell was so weak, that other ingredients overruled the smell of the basil in the control freeze dried pesto.

Table 15 Comparison of sensorial properties of pesto made out of untreated and PEF treated freeze dried basil leaves to pesto made of fresh basil leaves

Sample	Color and appearance	Smell
PEF freeze dried pesto	50.1 % of the panelists stated: color/look of this sample closer to the fresh basil. (1 panelist stated: no difference) Brighter, more liquid (oil layer), darker than fresh, darker than control freeze dried pesto.	100 % of the panelists stated: smell of this sample closer to the fresh sample. Weak basil smell, basil smell more intense than in control, leafy fresh, cheesy.
Control freeze dried pesto	33.3 % of the panelists stated: color/look of this sample closer to the fresh. Lighter, slight oil layer.	Nutty, no basil smell detected.

The last pair of pesto samples to be assessed was the untreated and PEF treated air dried pesto. The comparison and description of the appearance and smell of these samples is shown in Table 16. The panelists stated that those samples were the worst when it comes to appearance and smell when comparing them to the previous samples. However, 100 % of the panelists agreed that the PEF air dried samples was closer to the fresh pesto in appearance and smell.

Table 16 Comparison of sensorial properties of pesto made out of untreated and PEF treated air dried basil leaves to pesto made of fresh basil leaves

Sample	Color and appearance	Smell
PEF air dried pesto	100 % of the panelists stated: color/look of this sample closer to the fresh basil. Darker, greener, more intense green than fresh.	100 % of the panelists stated: smell of this sample closer to the fresh sample. More basil smell, but not comparable to fresh, cheesy, nutty.
Control air dried pesto	Big separation of two layers, close to pesto made of commercial dried basil, yellow, brownish, more oily, dark particles.	Strong smell of cheese and olive oil.

Examination of taste attributes of three chosen samples

The description of the samples to be tasted is shown in Table 17. The untreated vacuum dried sample was described by the panelists as the pesto with the most intense basil taste. The panelists were asked to rank the comparison of the taste of the 3 samples examined, where 50 % of the panelists stated that the control vacuum dried pesto was the closest to the fresh sample. 33.3 % panelists found the PEF freeze dried sample to be the closest to the fresh sample and one panelist stated it to be the PEF air dried pesto. The variability in the description was also

seen in the definition of the sample which is the furthest away in taste from the fresh sample. 50 % of the panelists stated that it was the PEF freeze dried sample and the other 50 % defined it as the PEF air dried sample. These observations showed that the control vacuum dried samples was overall perceived as closer in taste to the fresh basil pesto than the other two samples.

Table 17 Comparison and description of taste of untreated and PEF treated pesto samples dried with different techniques to fresh basil pesto as a standard

Pesto sample	**Taste**
PEF freeze dried	Slight hint of basil detected, cheesy, nutty.
PEF air dried	Basil taste detected, smooth (less smooth than fresh), cheesy, taste remains for a little while, fresh.
PEF vacuum dried	Basil taste perceived (less intense than fresh), strong nutty and cheesy aftertaste.
Control vacuum dried	Clear basil taste that lasts, intense (still not as intense as fresh), taste remains longer than for fresh sample.

7 Discussion

7.1 Differences in drying time

There is a rather big difference in drying time dependent on the drying technique and the pre-treatment applied to obtain a product with more or less the same moisture content.

The reduction in drying time between the untreated and PEF treated air dried sample was already described by Kwao *et al.* (2016). It was explained that the PEF treatment causes an irreversible opening of the stomata facilitating the evaporation of the water throughout the drying process, whereas the untreated sample will close the stomata leading to longer drying times. These findings can be supported by the SEM images of the stomata (Figure 12), as it was shown that the stomata of the control sample closed during the drying process, but stomata of the PEF treated sample kept open. The SEM images of the vacuum dried samples also, showed closed stomata for the control sample and open stomata in the PEF treated sample.

The drying time of the control air dried sample is significantly longer than the one of the control vacuum dried sample, even if the vacuum drying occurs at lower temperatures. As described by Prothon *et al.* (2003), during vaccum drying the low pressure used results in a bigger gradient in vapor pressure between the sample and the environment than that achieved during air drying, causing an increased mass transfer of moisture out of the sample. Additionally, the boiling point of water reduces with reduced pressure (Volland, 2011), leading to a faster evaporation of the moisture once the vacuum is build up.

The mass transfer principle explained above can be as well applied to the freeze drying method, where the mass transfer takes place via sublimation. However, there was not a big difference in the freeze drying time between the untreated and the PEF treated sample, in contrast with the other techniques. The SEM images of the stomata (Figure 12) might be an explanation for this observation, as both samples are shown to keep their stomata open throughout the drying process. There was, however, a shorter drying time of 3 hours obtained for the PEF treated sample for freeze drying, a difference that is not fully understood. I suggest that during the PEF treatment cells which are electroporated will leach a small amount of their liquid content. Normally viable cells would try to regain these liquids before they reverse the electroporation (Gómez Galindo *et al*, 2008; Phoon *et al.*, 2008), but during the sample preparation for freeze drying it is believed that the cells do not have enough time to regain the liquid before being frozen. Therefore, more ice crystals formed during freezing will be easily available for sublimation. This is an interesting issue for future research.

The reason why the stomata kept open in the control freeze dried sample should be also further investigated. To the best of my knowledge, there are no studies investigating the reason for the stomata opening throughout the freeze drying process of leaves. Prothon *et al.* (2003) stated that during freeze drying no visible structural changes might occur, but there are microstructural modifications taking place due to the damage caused by the ice crystal formation during the freezing step. Microstructural changes in the guard cells might lead to the irreversible opening of the stomata throughout the drying process.

7.2 Influence of drying techniques and PEF treatment on the structure/cell collapse

SEM image examination

It the SEM pictures (Figure 11) was clearly seen that the different drying techniques had an influence on the extent of cell collapse.

Freeze drying resulted in products with the least cell collapse observed, no matter if untreated or PEF treated, as these samples did not undergo any heat damage during the drying process. Whereas the control air dried sample showed the biggest extent of cell collapse in comparison to all the other samples. The PEF treated air dried sample displayed a distinct lower cell collapse than its control, probably due to the significant shorter heat treatment. Therefore, less heat damage of the cells results in less cell collapse (Prothon *et al.*, 2003). The cell collapse of the vacuum dried samples show a different pattern, as the control sample is less collapsed than the PEF treated sample. This observation is not well understood and is material for further investigations.

Prothon *et al.*, 2003 described the rehydration as an accepted measure to indicate the damage of a plant tissue occurring during drying, where reduced drying times and so less injured cells with a lower extent of cell collapse should show better rehydration capacities.

Results showing that all the dried samples did not show any significant difference in rehydration capacity (Figure 15), may indicate that all suffered the same extent of cell damage during the drying process. This finding cannot be confirmed by the extent of shrinkage and the SEM pictures (Figures 9 and 10), which indicate a more preserved cell structure with less cell collapse for the freeze dried samples, followed by the control vacuum dried sample. Kwao *et al.* (2016) indicated significantly better rehydration capacity for PEF treated air dried samples, comparing them to their untreated control. These findings were made with products at a final moisture content of around 10 %, which leads to the assumption that products dried to a final moisture content of around 7.5 % undergo more significant cellular modifications. These damages might be so sever, that the rehydration capacity reached a minimum for all the samples.

One of the drawbacks with SEM is that only an image of the surface structure of the cells is made, the porosity and interior cell damage of the tissue is not assessed.

7.3 Influence of the drying technique and PEF treatment on color

As describe by Di Cesare et al. (2003) observed color changes after drying are caused by a degradation in chlorophyll a (blue green) and b (yellow green) and enzymatic browning reactions. A transfer from a bright green color to an olive brown color should be observed due to a loss in Mg^{2+}. Chlorophyll a is known to be more sensitive when it comes to heat treatment, which means that during conventional drying, products may lose some of their green color and become more yellow. This was well shown by the hue values of the air dried samples, whereas all the other samples showed a shift towards the blue green color, as they did not undergo heavy heat treatments (Table 7). It can be concluded that the freeze dried and vacuum dried samples resulted in a darker and greener color compared to the fresh samples, whereas the air dried samples resulted in darker and yellower colors, even though there were slight differences between the colors observed. These outcomes were confirmed by the sensory panel, which found the samples with the darker green color (for both dried basil and pesto) to be more appealing than the yellowish ones. None of the samples assessed by the sensory panel were described as brown, except for the commercial dried basil sample. These observations suggest that the commercial sample showed a bigger extent of cell collapse, as these damaged cells release products prone to enzymatic browning (Di Cesare et al., 2003).

7.4 Influence of drying technique and PEF treatment on essential oil gland preservation

Mäkinen and Pääkkönen (1999) described that aroma compounds can be volatile (smell) or nonvolatile (taste), where the volatile ones are very delicate when heat treated during the drying process (Mäkinen and Pääkkönen, 1999). This means that the drying process can easily cause a decrease in aroma compounds. When observing the SEM picture (Figure 13), there was no difference in extent of disruption of the essential oil cavities between the different drying techniques observed, but it was clearly seen that the PEF treated samples could preserve the essential oil glands, with exception of the freeze dried sample. It was observed, that the PEF treated air- and vacuum dried samples preserved essential oil glands better due to a shorter drying process, accordingly conserving the aroma compounds. As both of the mentioned PEF treated samples showed this capacity is was assumed that the essential oil glands of the freeze dried samples are damaged due to the ice crystal formation during the freezing process.

The findings of the SEM images (Figure 13) were confirmed by the sensory evaluation, as the PEF treated samples were detected to be more intense in smell and closer to the fresh basil by the majority of the panelists, with exception of the control vacuum dried pesto. When it comes to taste, the vacuum dried samples were described as most intense and closest to the fresh basil. Followed by the freeze dried samples, even though the SEM pictures display a higher extent of aroma compound loss, shown by deformed and destroyed essential oil glands, when comparing it to the PEF air dried sample.

7.5 Industrial implications of PEF as a drying pre-treatment

Products obtained by vacuum drying were shown to result in samples which can be used for both applications, as dried product and for basil production, resulting in products being closest to the fresh samples, when compared to the samples dried with other methods. The PEF treated sample showed better volatile aroma compounds in dried form and the control sample when mixed in oil (for pesto).

For the vacuum drying technique the implication of a PEF pre-treatment should be assessed depending on the application the basil leaves will be used in. For pesto production there was no need to introduce a pre-treatment step when it comes to sensorial properties, but a reduction in drying time might be profitable on the long run. The application of a PEF pre-treatment could be suggested, even though a larger extent of collapse was observed. But the better preservation of essential oil glands, resulting in a better aroma compound retention was assessed by 100 % of the panelists and this product was detected to be the closest to the fresh basil when compared with the taste of the other dried samples. The color of the vacuum dried samples is darker green than the fresh leaves, which is appreciated by the consumers.

The application of PEF pre-treatment prior air drying of basil leaves is very much recommended as the production cost will be reduced due to significantly lower drying times. Additionally, a product with less cell collapse and a better retention in aroma compounds, with a color closer to fresh basil will be produced.

For the freeze drying technique the application of a PEF treatment is not recommended. The PEF treated freeze dried samples showed only a little shorter drying time, with no concrete prove of a higher aroma compound retention even though the PEF treated sample was recognized by the majority of the panelists to be closer in smell to the fresh basil than the

control. The cell collapse was as well greatly prevented in both untreated and PEF treated freeze dried samples and the color for both samples was a darker green than the fresh basil leaf.

8 Conclusions

- The quality properties of dried basil are influenced by the drying technique and the irreversible stomata aperture induced by pulsed electric field treatment.

- The different drying techniques and the PEF treatment showed an influence on drying time, where the PEF treatment reduced the drying time needed to reach the same amount of moisture content.

- The degree of cell collapse was shown to be different between the samples dried with different techniques, showing the least cell collapse in the freeze dried samples. The PEF treatment influenced the degree of cell collapse in both air dried and vacuum dried samples, with an exception in freeze dried samples. These cell collapse differences were assessed with SEM images, however, during rehydration experiments no significant differences between untreated and PEF treated samples dried with different techniques were observed.

- The essential oil gland preservation assessed with SEM showed that there was no influence of different drying techniques. However, PEF treatment significantly enhanced the preservation of essential oil glands in air- and vacuum dried samples with an exception in freeze dried samples.

- The sensory evaluation confirmed the positive influence of PEF treatment on aroma compound retention. With the PEF treated basils being the samples closer to the fresh standards with an exception for the pesto made from control vacuum dried leaves. Additionally, there was an influence on aroma compound retention between different techniques observed, where for both sensory evaluations the vacuum dried samples retained most aroma compounds, followed by the freeze dried samples.

- Vacuum dried samples were found to be closest to the fresh samples according to the sensory panel, when compared to the samples dried with other methods. The PEF treated sample showed better volatile aroma compounds in dried form and the control sample when mixed in oil (for pesto).

- An influence of the different drying techniques on the color of the leaves was observed. The air dried samples resulted in a yellower product when compared to the fresh sample and the vacuum and freeze dried samples changed towards a darker green. The sensory panel was able to clearly distinguish the color of the air dried samples with the samples dried with the other techniques. The PEF treatment showed a slight influence in the color change of the sample, but to a much lesser extent than the drying techniques. There was a rather clear difference between the untreated and PEF treated air dried sample, however, to distinguish between untreated and the PEF treated sample of the other two drying techniques demanded a very focused eye.

- The industrial implementation of PEF treatment prior to drying was suggested for air- and vacuum drying and was found to be inadvisable to freeze drying.

9 Future research opportunities

There is still room for a lot of different research topics to be investigated in this field. There were a few very interesting results resulting from this work, which I think would be worth it to investigate further.

Among these topics for further investigation, the cause for the lower drying time of PEF freeze dried samples in comparison to the untreated control (described in section 7.1), as both samples kept the stomata open throughout the entire drying process. Additionally, the investigation of the cause for enhanced cell collapse of PEF treated samples during vacuum drying (described in section 7.2) could be very interesting.

Another topic worth for investigation would be the production of aromatic edible oils, such as basil olive oil, through PEF treatment. The PEF treatment of basil leaves could be directly conducted within oil as a conductive media. By inducing irreversible permeabilization of the cell tissue, those applying PEF conditions in order to kill the cells, it should be possible to release the aroma compounds of the basil into the oil causing an uptake of aromatic basil flavor by the oil. This treatment would avoid alterations of sensorial properties by heat treatment, used in conventional extraction methods. A method should be found to increase the conductivity of the oil in order to make this theory work. As it was shown that olive oil for instance shows a very poor electric conductivity, with a high electric resistance. When heat was applied to the oil, a decrease in electric resistance was observed. (Lakrari *et al.*, 2013) But this would again cause sensorial alterations of the product due to heat treatment.

It can be seen that there is still a lot of room for investigation. The above suggested topics for investigation are only some of many possible, and I am looking forward to see the results of future studies conducted within this field in the coming years.

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12 Appendix

12.1 Pesto recipe

Table 18 Recipe for fresh basil pesto and pesto made with dried basil leaves

Ingredients	Dried basil pesto	Fresh basil pesto
Basil (g)	0,8	8
Pine nuts (g)	8,5	8,5
Parmesan cheese (g)	8,5	8,5
Olive oil (g)	20,5 (around 25 ml)	20,5 (around 25 ml)

12.2 Freezing curve

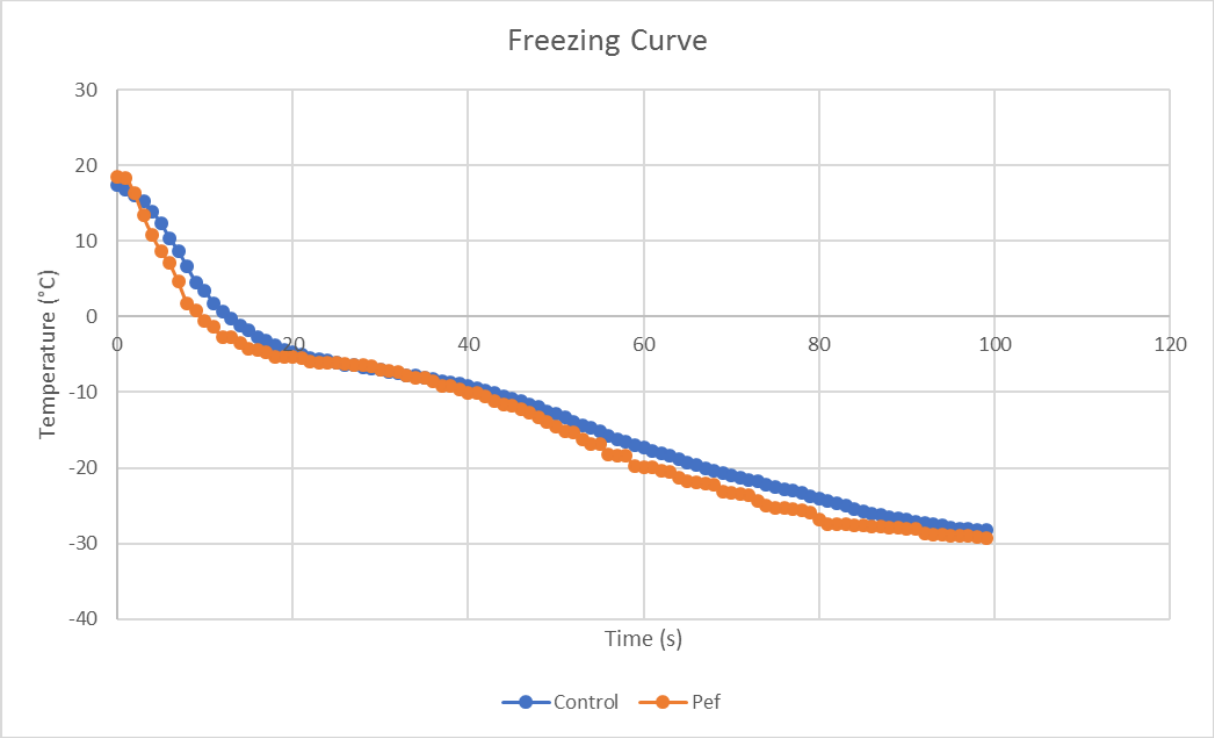

Figure 20 Freezing curve of untreated and PEF treated basil leaves to -30 °C