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PhD thesis

THE EFFECT OF PULSED ELECTRIC FIELD PRE-TREATMENT ON DRYING KINETICS AND QUALITY IN DEHYDRATED FRUITS AND VEGETABLES

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INDEX

To my beloved

grandfather

Index of figures

Index of tables

Index of flow diagram

SYMBOLS AND ABBREVIATIONS

E Electric field strength (kV/cm)	F Frequency (Hz)
n number of pulses	t drying time (min)
D effective diffusivity of water (m^2/s)	T air temperature (°C)
σ electrical conductivity (S /m)	m sample mass (kg)
CDI cell disintegration index	MC moisture content (%)
DR overall drying rate (g water/g dry solid min^{-1})	E_a activation energy (J mol^{-1})
M_0 initial moisture content	K specific rate constant
M_t moisture content at time t	PEF pulsed electric field
M_e equilibrium moisture content	L* brightness
mS/cm milliSiemens per cm	
Tt treatment time (ms)	a* red index
MR moisture ratio	b* yellow index
L half thickness of carrot & parsnip slices (m)	
R universal gas constant (J mol^{-1} K)	C* conductivities of untreated sample
T_p pulse width	H° hue angle
N_p number of pulses	DPPH 1,1-Diphenyl-2-picryl-hydrazyl
Q specific energy (kJ/kg)	D_{eff} effective diffusivity (m^2s^{-1})
V voltage (kV)	L half thickness
A current (A)	
m sample mass (kg)	

Subscript	**Description**
u	Conductivities of untretaed sample
t	Conductivities of treated sample
e_{ff}	Effective
low	Low frequency
high	High frequency

Abstract

The main objective of the thesis is to study the effect of different pre-treatments on the drying of vegetables foods. In particular, for Goji berries, classified as "super food", a traditional pre-treatment based on surface abrasion, was used. While for carrots and parsnips, an innovative pre-treatment, based on pulsed electrical fields, was used.

Goji berries have the epidermis covered by a thin layer of wax, which obstructs the passage of moisture from inside to outside the fruit. In this study a physical pre-treatment, consisting in a mild abrasion of Goji berry peel with a pilot plant, was considered. The main nutritional and sensory variations in goji berries were determined, such as color, total sugars, antioxidant activity and carotenoids.

The pre-treated berries showed reduced drying times and minor variations in color, while the sugar content was similar to untreated samples. In addition, the antioxidant activity of the abrasion-dried samples was higher than the untreated ones.

Pulsed electric fields (PEF) treatment is an innovative, alternative method to traditional cell membrane permeabilization. PEF treatment was performed in a laboratory plant. The electrical impedance measurement of untreated and PEF treated tissues was used to detect the degree of permeabilization of cell membranes.

The trials on carrot and parsnip slices have shown that the strength of the electric field and the number of pulses are the main process parameters that determine the effectiveness of the treatment. The degree of permeability is increased by increasing the electric field strength and the number of pulses. The study then investigated how the PEF pre-treatments influenced the drying kinetics and some qualitative parameters of the treated roots.

PEF pre-treatments significantly reduced the drying time of carrot and parsnip slices. The cell disintegration index (CDI) was significantly different between carrots and parsnips. The drying

time of carrots and parsnips treated with PEF was reduced to 21% at 60°C and 24% at 70°C, respectively.

PEF and high temperatures induced the Maillard reaction in the parsnips, with high production of furosine and darkening of the samples, confirmed by the decrease in brightness values (L*) and the significant increase in the values of the yellow index (b*). Dried carrots pre-treated with PEF showed a lower brightness value than untreated ones.

PEF treatment did not affect the characteristics of the texture of dried carrots and parsnips at 50 and 60°C, while a significant increase in the force needed to cut the slices at 70°C, was detected. Finally, the carotenoid content in dried carrots, mainly represented by β-carotene (69%) α-carotene (13%), phytoene (9%), 13-cis-β-carotene (8%) and 9-cis-β-carotene (1%), was more stable in PEF pre-treated samples.

CHAPTER I

1. INTRODUCTION:

Drying is one of the oldest food preservation method and one of the most commonly applied unit operation in food processing (Vega-Mercado *et al*., 2001; Lewicki *et al*., 2006; Y. Deng *et al*., 2008; S.K. Chou *et al*., 2001). The main aim of drying process is intended to remove water from foodstuff in order to prevent microbial spoilage and chemical alterations, thus prolonging shelf-life, while realizing space and weight saving (Cinquanta *et al*., 2010; Cuccurullo *et al*., 2012; Fratianni *et al*., 2013). Additional benefits of this process are related to lower shipping costs due to reduced mass and volume in comparison to a raw material. Unfortunately, convective drying is an energy intensive and consequently cost intensive method of food preservation (Rodrigues *et al*., 2008). In turn, high energy consumption is strictly connected with a high emission of substances that negatively impact the natural environment. Therefore, each activity that can possibly reduce the time of the process contributes both to costs reduction and to environmental protection. Moreover, shorter exposition of material on high temperature positively impacts the nutritional value of food. The reduction of drying time can be achieved either by technology alteration – for instance the swift from air drying to infrared drying – or by application of pre-treatment step (Lewicki *et al*., 2006; Nowak *et al*., 2004; Adedeji *et al*., 2008).

The goji fruit market today is significantly expanding, because of an increased awareness of their possible health benefits, as they are rich sources of micronutrients and phytochemicals, such as carotenoids, sugars, and phenolic compounds (Mikulic-Petkovsek *et al*., 2012a, 2012b). Goji berry is purported to benefit vision because of its high antioxidant (especially zeaxanthin) content. Some of these phytochemicals, which act as antioxidants, have recently been identified, and recent data show that they help to optimize human health by neutralizing free radicals in the body (Amagase *et al*., 2009; Donno *et al*., 2014).

Wolfberry has a specific epidermal structure covered by a thin layer of wax (Yang et al., 2011), like grapes and prunes (Adiletta *et al.*, 2016b; Cinquanta *et al.*, 2002). The epidermal wax inhibits moisture movement from the inside to the outside. For this reason, it is difficult to dry wolfberry. There is increasing interest in usage of chemical pretreatment, such as alkaline emulsion (mainly containing Na_2CO_3 and Na_2SO_3) dips for several minutes to dissolve the wax on the epidermis of wolfberry to enhance water diffusion through the epidermal wax layer (Wu *et al.*, 2015b; Li *et al.*, 2014). However, the chemical additive residue in the berries may cause food safety problems and how to deal with larger quantities of corrosive chemicals is a serious problem (Adiletta *et al.*, 2016b).

Conventional drying methods such as natural sun drying have been widely adopted for drying of wolfberry as it is cheap, but there is a risk of damage due to dust and insect infection. Furthermore, it requires large open areas, high labour costs and long drying time usually up to 4–5 days which is undesirable economically (Xie *et al.*, 2017). In general, disadvantages associated with hot air drying include damages in texture, color, taste and nutritional value of food due to the high temperatures and long drying times required in the process (Adiletta *et al.*, 2016a; Altimari *et al.*, 2011; Brasiello *et al.*, 2011, 2013; Russo *et al.*, 2012).

About the nutritional value, goji berries are an excellent source of carotenoids, not only because of their relatively high content in fruits, but also due to the specific profile of carotenoid species. During drying process, increasing temperature resulted in an oxidative degradation of pigment (e.g., carotenoids, astaxanthin and anthocyanin), which is positively correlated to the colour value. Therefore, a safer, efficient and suitable scheme for drying process of goji needs to be developed and applied to enhance quality attributes of wolfberry on a commercial scale. Drying combined with some pretreatments appears to be a cost-effective method of preservation. In this study, an alternative physical pre-treatment, consisting of abrasion of the peel of goji, was

proposed to reduce drying time and preserve the quality of final products (Di Matteo *et al.*, 2000; Cinquanta *et al.*, 2002).

Presently, a lot of attention has been paid to non-thermal pre-treatment methods of dehydration enhancement, for instance: high hydrostatic pressure (HHP), ultrasounds (US) and pulsed electric field (PEF) (Al-Khuseibi *et al.*, 2005; Rastogi *et al.*, 2000; U. Yucel *et al.*, 2010; Sledz *et al.*, 2013; Bantle *et al.*, 2011; Arevalo *et al.*, 2004; Wiktor *et al.*, 2014). Pulsed electric field (PEF) treatment is based on the application of very short, high voltage pulses to food placed between electrodes and leads to the electroporation, and, consequently, formation and growth of new and already existing membrane pores, disruption of the cell membrane and intracellular content leakage (Rastogi *et al.*, 1999; Knorr *et al.*, 2001; Ngadi *et al.*, 2003).

The application of pulsed electric fields to biological cells (plant or animal) mainly affects the cell membranes, inducing local changes in their structures and promoting the formation of pores. This phenomenon, named electroporation (or elecropermeabilization), causes a drastic increase in the permeability of cell membranes, which lose their semipermeability, either temporarily or permanently (Weaver & Chizmadzhed, 1996). The application of PEF as a permeabilization treatment to increase the rates of mass transfer of valuable compounds from biological matrices was demonstrated to be effective in drying, extraction, and diffusion processes.

CHAPTER II

2. LITERATURE OVERVIEW:

Many researchers in different countries investigated different drying processes for fruits and vegetables. This work studied the effect of abrasive and pulsed electric field (PEF) pretreatment on the drying of goji berry, carrot and parsnip by convective hot air drying under different combinations of temperature and pretreatments. The available information related to the present study is cited below.

2.1. Drying and quality

Dehydration changes food products in several ways, affecting the organoleptic qualities of the product. Dehydration normally requires high temperatures, which can cause chemical reactions such as non-enzymatic browning, caramelization, and denaturation of proteins in the product. Drying also affects the physical parameters of the product, as removal of water causes shrinkage (plums become prunes, grapes become sultanas). Due to these changes, rehydration after drying may not restore the original product.

The basic configuration of an atmospheric dryer is a chamber in which the food is introduced and equipped with a fan and pipes that allow the circulation of hot air through and around the food. Water is eliminated from the surface of the food and driven outside the dryer, with the air stream that leaves it, in a simple operation. Air is heated at the inlet of the dryer by heat exchangers or directly with a mixture of combustion gases. This type of dryer is widely used in the production of cookies, dried fruits, chopped vegetables, and food for domestic animals. The drying rates of food materials are usually determined experimentally, since it is very difficult to predict accurately the heat and mass transport rates on purely theoretical grounds. The drying tests are normally carried out on a layer of material, placed in an experimental dryer, which is operated under controlled conditions of temperature, air velocity, and humidity.

The drying rate curve may indicate a constant rate period, during which mass transfer from the surface of the material controls the drying process, depending mainly on the external conditions (air velocity, temperature, and humidity). Short constant drying rates may be observed in air-drying food materials of high moisture content or in washed products, containing free surface water. However, most food materials do not show any constant rate and they dry entirely in the falling rate period, during which mass transfer is controlled by the transport (diffusion) of water through the material to the surface of evaporation. Dried and dehydrated foods are generally microbiologically stable, i.e., microbial growth is prevented by the low water activity *(aw* < 0.70).

2.2. Characterization of goji berry, a superfood

Superfruits, a subcategory of superfoods, is a relatively recent word and is considered a new marketing approach to promoting common or rare fruits which can be consumed as foodstuffs or used as ingredients by manufacturers of functional foods, beverages and nutraceuticals. Superfruits have a high nutritional value due to their richness in nutrients, antioxidants, proven or potential health benefits and taste appeal (Felzenszwalb, 2013). In the functional foods market, the products targeting health and mental well-being have prompted the food industry to increase the research and the development of these new foods, outlining a rapid expansion market in several countries (Vicentini *et al.*, 2016). In the last years, goji berries have been cultivated in Italy and are available both as fresh and dried fruits. While several papers on the medical effect of goji berries have been published, little information is available on the nutritional composition of dried and, above all, fresh goji berries.

Fruits of *Lycium barbarum* L., belonging to Solanaceae family, commonly known as goji berries or wolfberries, have been used in Chinese traditional medicine for centuries. *Lycium barbarum* grows in China, Tibet and other parts of Asia and its fruits are 1–2 cm-long, bright orange-red ellipsoid berries. The native area of Lycium is not definitively established but it is likely found in the Mediterranean Basin (Potterat, 2010).

Traditionally, goji berries are collected in summer and autumn. The fruits can be eaten fresh or dried, and they are also found in conventional food products, such as yoghurt, fruit juices, bakery foods, chocolate and others (Mikulic-Petkovsek *et al*., 2012a, 2012b). Traditionally, the berries are dried in the shade until the skin shrinks and then exposed to the sun until the outer skin becomes dry and hard but the pulp is still soft (Amagase and Farnsworth, 2011**)**. The sun drying method is cheap, but there is a risk of damage due to dust and insect infestation. An alternative is hot air drying. Today the goji fruit market is significantly expanding because of an increased awareness of the possible health benefits, as fruits contain different nutrients, such as polysaccharide complexes, organic acids, phenolic compounds and antioxidants with high biological activity. Dietary fibre provides several health benefits, including the reduction of the risk of coronary heart disease, of diabetes, hypertension, obesity, stroke and some gastrointestinal disorders (EFSA, 2010). Recent studies indicate that polysaccharides from *Lycium barbarum* possess a range of biological activities, including antioxidant properties (Amagase and Nance, 2008; Chang and so, 2008). Goji fruits contain ascorbic acid and different carotenoids (Kulczyński and Gramza-Michałowska, 2016). Carotenoids are a significant group of biologically-active constituents with health promoting properties (Amagase *et al*., 2009; Donno *et al*., 2014), responsible for the colour of a wide variety of foods (fratianni *et al*., 2005).

The reddish-orange colour of *L. barbarum* fruits derives from a group of carotenoids, which make up only 0.03–0.5 % of the dried fruit. Zeaxanthin is the major carotenoid found in Goji.

This is a yellow pigment, an isomer of lutein and a derivative of β-carotene. When ingested, zeaxanthin accumulates in fatty tissues, but especially in the macula, a region of the retina, helping in protecting the macula from degeneration, which can be induced by excessive sun exposure (UV light) and by other oxidative processes (Cheng *et al*., 2005; Rosenthal *et al*., 2006; Trieschmann *et al*., 2007). In Goji, zeaxanthin is present as an ester of dipalmitate. Studies focusing on carotenoid goji berries are few and mainly aimed at the identification and quantification of ester-form carotenoids. (Inbaraj *et al*., 2008 and Zhao *et al*., 2013), in particular, identified free-forms and ester-forms of carotenoids. Beta-carotene, neoxanthin, and cryptoxanthin are also present at low concentrations (Peng *et al*., 2005; Wang *et al*., 2010). Regarding other antioxidants, studies made on *Lycium chinense Miller* reported high amounts of α- tocopherol**,** together with other vitamin E compounds (Isabelle *et al*., 2010). Vitamin E is a generic term indicating structurally related compounds, namely tocols, comprising two groups of vitamers, i.e. tocopherols and tocotrienols, which occur in eight forms: α-tocopherol (α-T), β–tocopherol (β-T), γ-tocopherol (γ-T), and δ-tocopherol (δ-T) and α-tocotrienol (α-T3), β-tocotrienol (β-T3), γ-tocotrienol (γ-T3), and δ-tocotrienol (δ–T3). The potential health benefits of tocols have been the subject of several reviews (Tiwari and Cummins 2009). Vegetable oils are the main tocol source; however, substantial amounts of these compounds are also reported in most cereal grains (Fratianni *et al*., 2013, Mignogna *et al*., 2015). To our knowledge, no literature data are available on the composition and content of tocols in *L. barbarum* fruits. Goji is also an extremely rich source of many essential minerals, which are essential for many actions in the body, like muscle contraction, normal heart rhythm, nerve impulse conduction, oxygen transport, oxidative phosphorylation, enzyme activation, immune functions, antioxidant activity, bone health, and acid-base balance of the blood (Williams, 2005). An adequate daily amount of minerals is necessary for an optimal functioning of the body. For the above-reported reasons, goji berries are often proposed as functional foods and

have been included in the novel category of "superfruits" or "superfoods". In last years, goji berries have been cultivated in Italy and are available both as fresh and dried fruits. While several papers on the medical effect of goji berries have been published, few information are available on the nutritional composition of dried and, above all, fresh goji berries.

2.3. Characterization of carrot and parsnip

Carrot (*Dacus carota* L.) and parsnip (*Pastinaca sativa* L.) are roots that belong to the Apiaceae family. Carrot is one of the important root vegetables rich in bioactive compounds like carotenoids and dietary fibers that is approximately 20% of the dry matter (Bengtsson and Tornberg, 2011), about 9% as insoluble pectin. Parsnip roots have a relevant Total Antioxidant Capacity: 1000-3000 µmol of Trolox Equivalent per 100g (Pennington and Fisher, 2009). Fibers with high content of insoluble pectin will be more resistant to disruption than fibers with low content of insoluble pectin (Bengtsson and Tornberg, 2011). The degree of susceptibility of the cell wall material to be disrupted could to be an important factor for choosing and set PEF drying pre-treatments. For instance, the difficulty to break and disintegrate parsnip cell walls could be related to its relatively high content of insoluble pectin, present in the cell walls and in the middle lamella (Coutate, 2009). The quality of carrots and the high content of dietary fiber in parsnip makes both roots very attractive for industrial use with nutritional purposes (Figure 2.).

2.4. Methods of cell membrane disintegration in food processing

Cell membrane disintegration is defined as the irreversible breakdown of the cell membrane in order to increase its permeability and/or cause cell death (lysis). The disintegration of the cell membrane is used in many fields such as biotechnology, cell biology, medicine and food industry. Mass transfer processes such as solid-liquid extraction and drying as well as food

preservation are important unit operations of the food industry requiring the disintegration of the cell membrane.

When the cell membrane of a microorganism is broken, cell lysis takes place and the inactivation of the microorganism occurs. This is the way to achieve food preservation and extending the shelf life of food products inactivating pathogens responsible of undesired food degradation.

On the other hand, cell membrane acts as a physical barrier in removing the intracellular substances (water, juices and solutes) from plant food tissues in solid-liquid extraction and drying. Thus, the disintegration or permeabilization of the cell membrane in a plant food tissue causes the release of intracellular water and solutes (secondary metabolites) to migrate in an external medium. This can be seen as an effective tool to promote mass transport processes, so that higher yields and shorter contact time can be achieved in the above mentioned unit operations.

According to these applications, the degree of the cell membrane disintegration is very important determining the efficiency of the process. Presently, the rupture of the cell membrane can be obtained by means of several methods according to the desired degree of disintegration and to the particular application. It is possible to identify: thermal and non-thermal methods.

High temperature is used in food preservation (pasteurization/sterilization) and in pre-treatment and/or complementary stages before extraction processes (sugar beet processing) and drying (hot water blanching). In this way it is possible to achieve a high degree of cell membrane breakdown.

2.5. Pulsed electric field

PEF treatment involves the application of repetitive ultra-short pulses (from ns to μs) of a high-strength electric field (0.1-10 kV/cm) through a material located between two electrodes. The

application of the external electric field induces the permeabilization of cytoplasmatic membranes. The main advantages of PEF with respect to other treatments addressed to disrupt the cell membranes, such as the application of heat or the addition of pectolytic enzymes, are as follows:

- Cost reduction due to lower energy consumption and unnecessary enzyme addition
- Higher purity of the extracts, since upon the PEF treatment the permeabilized cell membranes maintain their structural integrity and are not disrupted in small
- Lower processing times thanks to the increased mass transfer rates.

This chapter reviews the basic mechanisms of PEF-induced permeabilization of plant tissues, discusses the methods of detection of electrically induced cell damages and analyses the influence of PEF process parameters on mass transfer. Furthermore, mathematical models to describe the mass transfer rates from PEF-treated vegetable tissue are discussed and some criteria of energy optimization are given as well as some examples on the recovery of polyphenolic compounds from food matrices and on the integration of PEF treatments in the winemaking industry.

Electroporation is today widely used in biotechnology and medicine to deliver drugs and genes into living cells (Neumann et al., 1982; Fromm et al., 1985; Mir, 2000; Serša et al., 2003; Miklavčič et al., 2006). Recently, the interest in electropermeabilization has considerably grown, as it offers the possibility to develop different non-thermal alternatives to the traditional processing methods of the food industry requiring the disintegration of cell membrane. For example, the complete damage of the microbial cell membrane induced by the application of intensive PEF process conditions has been intensively studied in the last twenty years as a new nonthermal method of food preservation (Barsotti and Cheftel, 1999; Mosqueda-Melgar et al., 2008; Pataro et al., 2011). More interestingly, it has been also reported by several research teams that the application of a pulsed electric fields pre-treatment of moderate intensity to

biological tissue may considerably increase the mass and heat transfer rates between plant cells and the surroundings, making it suitable for enhancing the efficiency of the pressing, extraction, drying and diffusion processes of the food industry (Angersbach, 2000; Vorobiev et al., 2005; Vorobiev and Lebovka, 2006; Donsì et al., 2010b).

If a critical value of the field strength *Ec* is exceeded, a critical trans-membrane potential can be induced (typically 0.2-1.0 V for most cell membranes) that leads to the formation of reversible or irreversible pores in the membrane (Zimmermann and Neil, 1996). The occurrence of reversible or irreversible permeabilization of the cell membranes depends on the intensity of the external electric fields, pulse energy and number of pulses applied. The greater the value of these parameters, the higher is the extent of the membrane damage (Angersbach et al., 2002). When a mild PEF treatment is applied, either because the electric field applied is below the critical value *Ec* or the number of pulses is too low, reversible permeabilization occurs, allowing the cell membrane to recover its structure and functionality over time. On the contrary, when more intense PEF treatment is applied, irreversible electroporation takes place, resulting in cell membrane disintegration as well as loss of cell viability (Zimmerman, 1986).

The range of characteristic low and high frequencies used depends on the cell size in relation to the conductivity of cell liquid and neighboring fluids, as shown in (Table 1.) (Angersbach et al., 2002).

According to electroporation theory, the extent of cell membrane damage of biological material is mainly influenced by the electric treatment conditions. Typically, electric field strength E, pulse width τ_p and number of pulses n_p (or treatment time $t_{PEF} = \tau_p \cdot n_p$) are reported as the most important electric parameters affecting the electroporation process. In general, increasing the intensity of these parameters enhances the degree of membrane permeabilization even if, beyond a certain value, a saturation level of the disintegration index is generally reached (Lebovka et al., 2002). Theoretically, the total cell permeabilization of plant tissue was

obtained by applying either one very high energy pulse or a large number of pulses of low energy per pulse (Knorr and Angersbach, 1998). A PEF system for food processing in general consists of three basic components: a high voltage pulse generator, a treatment chamber and a control system for monitoring the process parameters.

The electrically induced perforation of the cell membrane may enhance drying, as it alters the cellular structure of the plant material which is considered as one of the drying limiting factors (Figure 3.) . Numerous scientific data indicate that PEF application improves both dehydration processes and changes in some properties of dehydrated food (Shynkaryk *et al.*, 2008; Gachovska *et al.*, 2009; Grimi *et al.*, 2010), including the electrical conductivity, thus the efficiency of electroporation can be monitored by an electrical conductivity measurement (Bazhal *et al.*, 2003). In considerations also of the differences in the structure of carrot and parsnip, it was decided to evaluate the effect of pre-treatments with PEF on the kinetics of drying and on the quality of the two roots, by evaluating color and textural changes.

2.5.1. Detection and characterization of cell disintegration in vegetable tissue

The early attempts in studying the degree of cell permeabilization was based on quantifying the release of intracellular metabolites (i.e. pigments) from plant cultured cells after electroporation induced by the application of Pulsed Electric Field (Brodelius et al., 1988; Dörnerburg and Knorr, 1993). The irreversible permeabilization of cells in vegetable tissues has been demonstrated, for the first time on potato tissue (exposed to PEF treatment), determining the release of the intracellular liquid from the treated tissue using a centrifugal method.

CHAPTER III

3. OBJECTIVES OF THE STUDY

The main objective of the thesis is to study the effect of different pretreatments on the drying of plant products. In particular, it was used for a "super food", Goji berries, a traditional pretreatment based on surface abrasion. While in the case of carrots and parsnips, an innovative pretreatment was used, using pulsed electrical fields.

Furthermore, to determine the compositional and nutritional value of fresh and dried goji berries (*Lycium barbarum L.*) cultivated in Italy, with a particular focus on colour, sugar content, minerals and some antioxidant compounds, such as carotenoids and tocopherols, so that to increase the awareness about their nutritional profile.

Moreover, we evaluated the effect of PEF pretreatment, carried out at different parameters, on the color and microstructure changes of a convectively dried carrots and parsnips. . The study then investigated how the PEF pre-treatments influenced the drying kinetics and some qualitative parameters of the treated plants.

On the basis of the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities

A1) Quality analysis

- Proximate composition analysis.

A2) Sensorial analysis

- Color analysis by colorimeter.

A3) Nutritional analysis

- Carotenoid analysis by HPLC.
- Mineral analysis.

- Tocopherol analysis.
- Antioxidant analysis.
- Sugar analysis.

A4) Estimation of the process efficiency comparing the drying kinetics of untreated and treated goji berry samples in convective hot air oven.

A5) Statistical analysis done by analysis of variance (ANOVA) method (using an SPSS version 13.0 for Windows).

B1) Quality analysis

- Proximate composition analysis.
- Cell disintegration index measurement
- Color analysis by calorimeter.
- Texture analysis by universal testing machine.

B2) Nutritional analysis

- Carotenoid analysis by HPLC.
- Total soluble phenolics (TSP) analysis

B3) Thermal damage analysis

- Furosine content

B4) Energy consumption

B5) Microscopic images by optical microscope.

B6) Estimation of the process efficiency comparing the drying kinetics of untreated and PEF treated carrots and parsnip samples in tray drier.

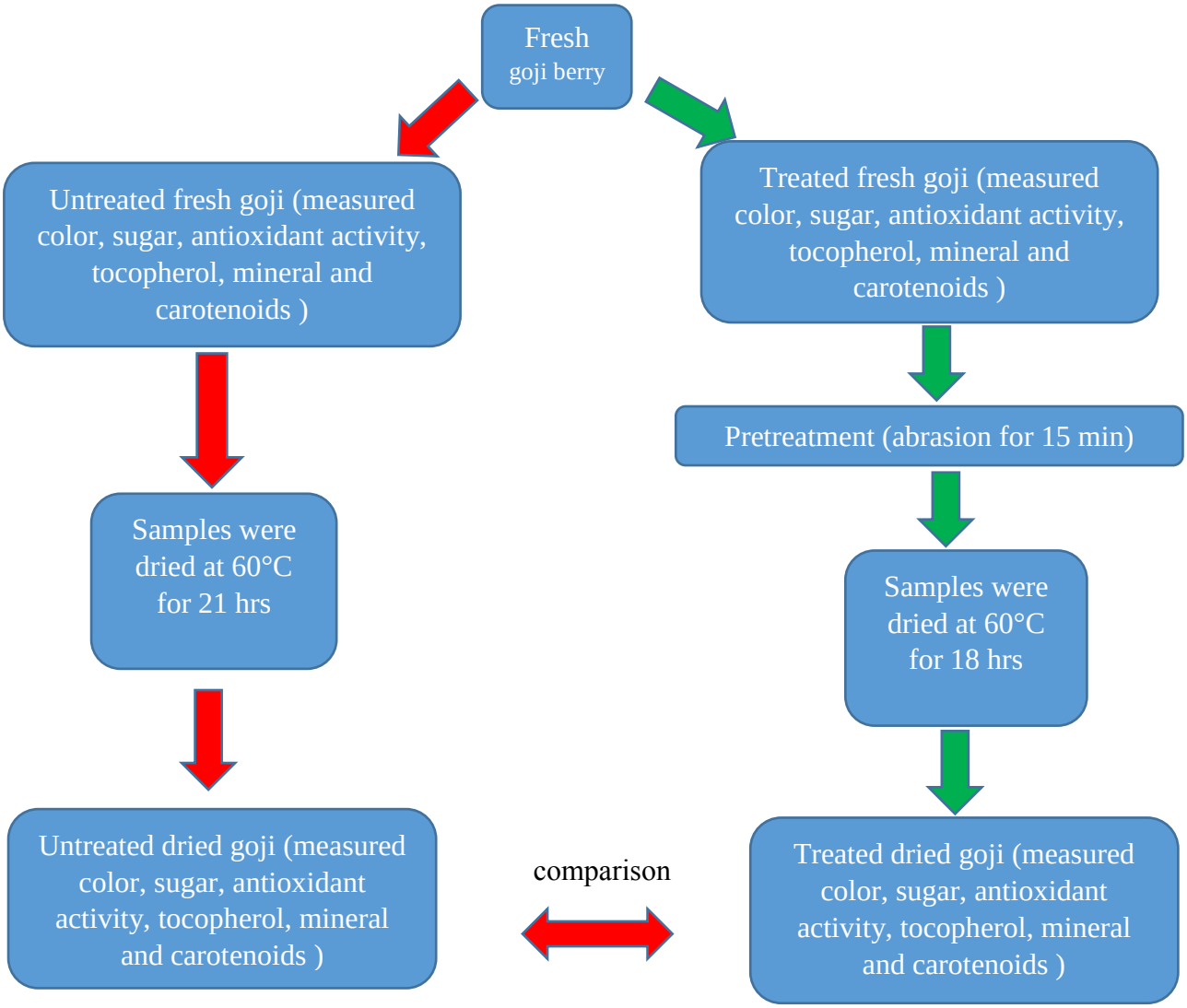
B7) Statistical analysis done by analysis of variance (ANOVA) method (using an SPSS version 13.0 for Windows).

CHAPTER IV

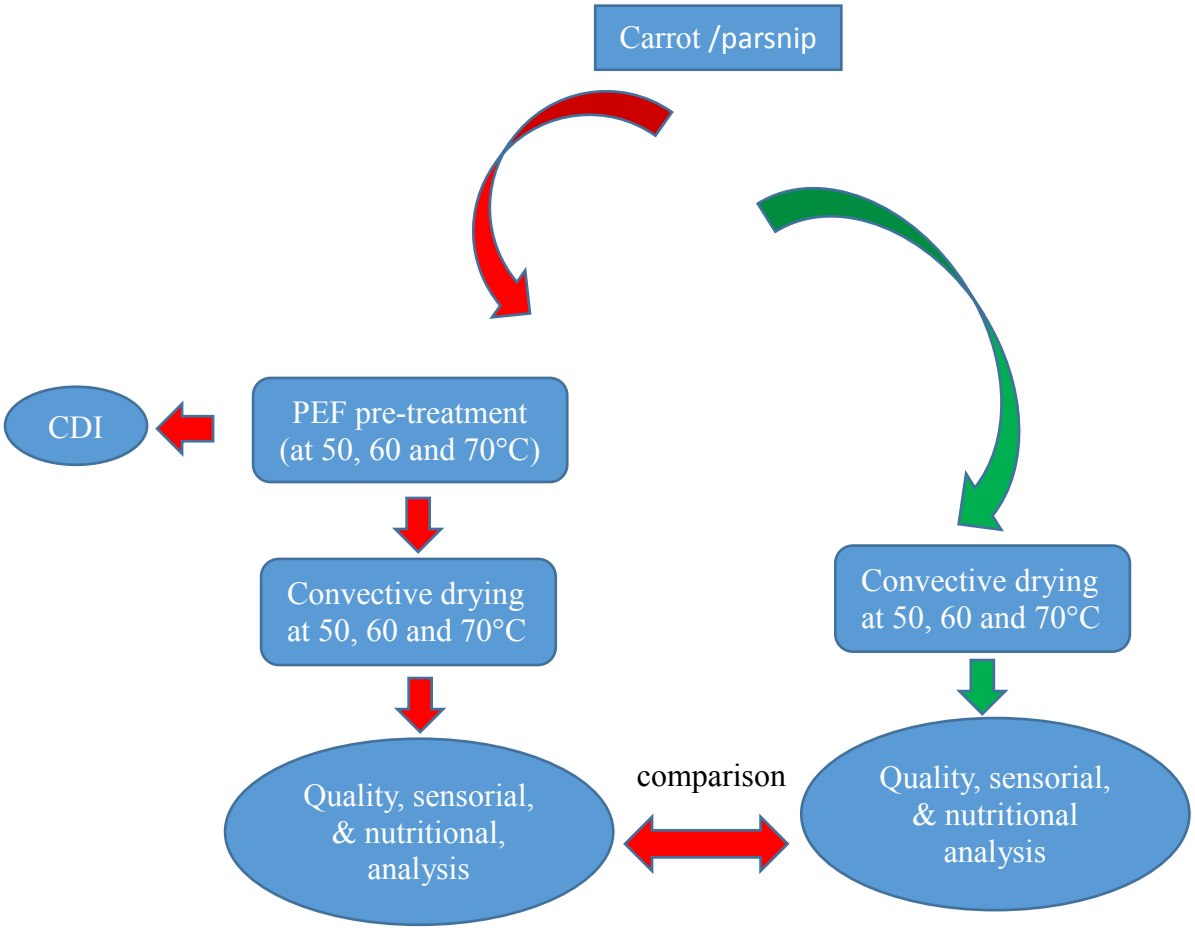
MATERIALS AND METHODS

4.1. METHODOLOGY

2. Flow diagram: Methodology for goji berry.



2. Flow diagram: Methodology of carrot and parsnip.



4.2 MATERIALS AND METHODS

4.2.1. Sample collection and preparation

Fresh goji berries (*L. barbarum L.*) were provided by *Favella Spa farm* (Sibari, Southern Italy). The farm has 21000 plants in 5 Ha (2.5 m x 1 m), with a drip irrigation system. Goji berries were cultivated in two consecutively growing seasons (2014 and 2015) and were collected in July. All harvested fruits were randomly collected in the orchard from different plants and analysed fresh and air-dried. Fresh goji berries (about 1 cm size) were subjected to freeze-drying before analyses, as reported by (Fratianni *et al.*, 2013) (fresh fruits). One-half of collected goji berries were air-dried in a convective dryer (B80FCV/E6L3, Termaks, Norway) (Figure 6), at 60 °C, with an air velocity of 2.1 m/s, until a constant weight was reached (dried fruits) (Adiletta *et al.*, 2015).

4.2.2. Proximate composition analysis (goji berry)

Fresh and dried fruits were analysed for moisture, ash, fat, and protein (N×6.25) contents, according to standard methods of (AOAC, 2000). Dietary fibre content was determined according to (AOAC method 991.43, 1995) and (AACC method 32-07, 1995). Total dietary fibre content was the sum of insoluble and soluble dietary fibre content. Vitamin C was determined by using an enzymatic kit (Megazyme International, Ireland), following the manufacturer instructions.

4.2.3. Quality analysis of goji berry

Mineral analysis

Ultrapure nitric acid for trace analysis, sulfuric acid (96 %) and standard mono elements in nitric acid 2 % were purchased from Sigma-Aldrich (20151 Milan, Italy). The determination of metals (K, Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, P, Se, Zn) in goji samples was carried out by

using the technique of nitric mineralization and the analysis by spectrophotometry plasma emission (*Varian ICP 710, OES* Inductively Coupled Plasma-Optical Emission Spectrometers, Palo Alto, CA 94304-1038). Samples were ground and 0.5 g was digested with 10 ml of nitric acid with a mineralizer (SCP Science DIGIprep, Quebec H9X 4B6, Canada), with the following instrumental conditions: start at 40 °C for 15 min; heating at 60 °C for 15 min; stay at 60 °C for 15 min; heating to 90 °C for 20 min. The digested samples were cooled and brought to a volume of 50 ml with bidistilled water and analysed with the optical ICP. The precision was calculated as a mean deviation of three measurements.

Carotenoid analysis

Extraction and determination

Carotenoid extraction was carried out using the direct solvent extraction method reported in (Fratianni *et al.*, 2013), with slight modifications, due to the complex structure of goji berries. About 0.1 g of milled freeze-dried samples (fresh fruit) and air dried samples (dried fruit) was weighed and placed in a screw-capped tube. Then, 5 ml of ethanolic pyrogallol (60 g/L) was added as an antioxidant. The sample was stirred for 10 min. After that, 2 ml of absolute ethanol was added and sample was stirred again for a few min. The suspension was then extracted with 15 mL of n-hexane/ethyl acetate (9:1 v/v) and stirred; after that 15 mL of sodium chloride (10 g/L) was added. Further extractions with n-hexane/ethyl acetate (9:1 v/v) were made, until the organic layer was colourless. Finally, the organic layer was collected and evaporated to dryness, and the dry residue was dissolved in methanol: MTBE 50:50 (v/v). This sample was used to determine the free carotenoids, not esterified with the lipid components, and carotenoids esterified with fatty acids (unsaponified). A volume of 2 ml of this extract was evaporated to dryness and subjected to alkaline hydrolysis, under a nitrogen flux for 1 minute, in a screw-capped tube, with 1 ml of ethanolic pyrogallol (60 g/L), added as antioxidant, 10 ml

of solvent HEAT (hexane: ethanol: acetone: toluene 10: 6: 7: 7 v/v/v/v), 2 ml of methanolic KOH (40 %) and glass balls. The tubes were placed in a 56°C water bath and mixed every 5 to 10 min. After alkaline digestion at 56 °C for 20 min. the tubes were cooled in an ice bath, and 15 mL of sodium chloride (10 g/L) were added. The suspension was then extracted with 15 mL of n-hexane/ethyl acetate (9:1 v/v) until the organic layer was colourless. The organic layer was collected and evaporated to dryness, and the dry residue was dissolved in methanol: MTBE 50:50 (v/v). This sample was used to determine carotenoids esterified with lipid components (saponified). An aliquot of the carotenoid extract was separated, as in, by a reverse-phase HPLC system. An HPLC Dionex (Sunnyvale, CA) analytical system, consisting of U3000 pumps, and an injector loop (Rheodyne, Cotati) were used. Separation was performed, as in (Fratianni *et al.*, 2013), by using an YMC (Hampsted, NC, USA) stainless steel column (250×4.6 mm i.d.), packed with 5 µm silica spheres that were chemically bonded with a C30 material, at a flow rate of 1 mL/min. The mobile phase was methanol: MTBE (v/v). The eluted compounds were monitored by a photo-diode array detector (Dionex, Sunnyvale) set at 430 nm.

Identification and quantification

Carotenoids were identified on the basis of diode array spectral characteristics, retention times, and relative elution order, compared with known commercially available standards. All-trans-β-carotene and lutein were from Sigma Chemicals (St. Luis, MO, USA), zeaxanthin and β-cryptoxanthin were obtained from Extrasynthese (Z.I. Lyon-Nord, Genay, France). Zeaxanthin dipalmitate was identified by means of spectral characteristics found in literature (Inbaraj *et al.*, 2008). Compounds were identified by comparison of their retention times with those of known available standard solutions and quantified on the basis of calibration curves of standard solutions. Zeaxanthin dipalmitate was quantified as zeaxanthin.

Tocopherol analysis

Tocols were determined after the saponification method of the extract described for carotenoids. An aliquot of the carotenoid extract was collected and evaporated to dryness, and the dry residue was dissolved in 2 ml of isopropyl alcohol (1 %) in n-hexane, and was analysed by HPLC, under normal phase conditions, using a 250 x 4.6 mm i.d., 5 mm particle size, and Kromasil Phenomenex Si column (Torrance, CA, USA) (Panfili *et al.*, 2003). Fluorometric detection of all compounds was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm, by means of an RF 2000 spectrofluorimeter (Dionex, Sunnyvale, USA). The mobile phase was n-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 v/v/v) at a flow rate of 1.6 mL/min (Panfili *et al.*, 2003). Compounds were identified by comparison of their retention times with those of known available standard solutions and quantified on the basis of calibration curves of standard solutions. The concentration range was 5-25 µg/ml for every tocol standard. Vitamin E activity was expressed as Tocopherol Equivalent (T.E.) (mg/100 g product), calculated as reported by (Sheppard *et al.*, 1993).

Color measurements

Color was obtained through a colorimeter Minolta Chroma Meter II Reflectance CR-300 (triple flash mode aperture 10 mm). In order to understand the colour change of all samples (fresh and dried), CIELAB L*, a* and b* colour coordinates were recorded. It was recorded using CIE L*a*b* uniform color space (CIE-Lab). The lightness value (L*) indicates the darkness/lightness of the sample, a* is a measurement of the greenness/redness of the sample and b* is the extent of blueness/yellowness. Chroma (C*) and Hue angle (H°) were calculated as follows:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$H° = tan^{-1} b^*/a^* \quad (2)$$

Chroma indicates the dullness/vividness of the product while the Hue angle is how an object's color is perceived by human eye: red, orange, green or blue.

Antioxidant activity measurement

The antioxidant activity was evaluated by the DPPH scavenging method (Song and Xu, 2013). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant, its absorption decreases.

The absorbance of fresh and dried samples was measured against the blank at 517 nm by a spectrophotometer (Perkin Elmer, Lambda Bio 40) at room temperature. 100µl of the extract in methanol was added in a cuvette containing 2.9 mL of methanol solution of DPPH (0.1mM). The solution in the cuvette was shaken well and incubated in the dark for 60 min at room temperature.

Applying the same procedure to a solution without the test material a blank experiment was carried out; the absorbance was recorded as A_{blank}. The antioxidant activity was expressed as the percentage inhibition of DPPH (Albanese *et al.*, 2013, 2014) and then calculated according to the following equation:

$$\text{Inhibition of DPPH } (\%) = 100 * \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \quad (3)$$

The analysis of percentage inhibition of DPPH was replicated three times. The results obtained were reported as % inhibition of DPPH/mg db (dry basis).

Sugar analysis

The Sugars (fructose, glucose and trehalose concentrations) were determined by HPLC in a Waters 600 apparatus (Milford, MA, USA) with a refractometric detector (Waters 470), and with a 300×4 (id) mm column Sugar-Pak-(Waters), at 85 °C; the flow rate of the eluate was 0.6 ml/min (Di Matteo *et al.*, 2003).

Statistical analysis

The means and standard deviations of experimental results were calculated from three replicates. One-way ANOVA (analysis of variance) at the level of significance $p < 0.05$ using Tukey test was performed for comparison of means.

4.2.4. Pretreatment

Pretreatment was done to destroy the resistance of waxy layer which prevents moisture transfer and increases the drying rate. Before drying, treated abrasive (TR-Abr) samples were submitted to a physical abrasive pretreatment.

A patent is applying for this new system. The abrasion of the goji peel was carried out in a pilot system with motorized rotating drum created by prof. Marisa Di Matteo, Department of Industrial Engineering, University of Salerno (Di Matteo *et al.*, 2000; Cinquanta *et al.*, 2002). The drum was made of plexiglass, lined inside with sandpaper. The rotation speed of drum was 9 rpm, the pretreatment time was 15 min (Figure 4).

4.2.5. Proximate composition analysis (carrot and parsnip)

Fresh and dried fruits were analysed for moisture, ash, fat, and protein (N×6.25) contents, according to standard methods of (AOAC, 2000).

Measurement of the cell disintegration index

Sample preparation for measuring the cell disintegration index

Raw carrots (*Daucus carota*) and parsnips (*Pastinaca sativa*) were purchased at a local supermarket and stored at 4°C. Before undergoing measurement of cell disintegration index (CDI), produces were taken from the refrigerated store and were allowed to equilibrate at room temperature (20±1°C). Roots were cut by using a corer into a cylinder shape of 35 mm diameter (with the axis along the direction of the root growth) and a suitable height to reach the weight of 35 g. A treatment chamber having two stainless steel parallel plate electrodes of 16 cm^2 area separated by a gap of 4 cm was used (Aguiló-Aguayo *et al.*, 2014). The cylinder was placed inside the chamber with the circular surfaces facing the electrodes. A salt solution of 0.3 mS/cm was added into the chamber in order to simulate the conductivity of tap water, the salt solution was prepared weighting 304.7 mg of KCl into a 2L flask according to the International Recommendation No.56, International Organization of Legal Metrology 9OIML. The sample was kept in a thermostatic bath (Mod. JB5, Grant Instruments Ltd, Cambridge, UK) at 25 °C for 45 min in order to reach a homogeneous temperature of 25°C.

PEF treatment before the cell disintegration index measurement

A lab scale pulsed electric fields (PEF) unit (ELCRACK HVP5, DIL, German Institute for Food Technologies, Quakenbrück, Germany) with a maximum output voltage of 25 kV was used. The machine provides bipolar near rectangular-shaped pulses. The PEF treatment was performed setting the pulse width of 20 μs, the frequency of 50 Hz and the output voltage of

23%. A PicoScope 2205 MSO oscilloscope connected to the transformer (one meter cable from the treatment chamber) was used to record the pulses. An example of a positive pulse was shown in (Figure 7) where 1V = 1 kV (red line) and 1A = 20 A (blue line). The average and standard deviation of five replicated of electric field strength (EFS) and peak current values of carrots and parsnips were recorded from the result section of the machine after each treatment are reported in the (Table 2) and (Table 3) respectively.

4.2.6. Cell Disintegration Index estimation

A valuable method to estimate the cell disintegration index (CDI), also known as degree of tissue damage, consists in measuring the value of electrical conductivity of the tissue at suitable low and high frequencies before and after the PEF treatment (Angersbach *et al.*, 1999). The CDI goes from 0, for an intact tissue, up to 1, for a maximally damaged tissue. The suitable low and high frequencies selected in this study were 0.5 kHz and 20 MHz respectively (Angersbach *et al.*, 2002). In this study the CDI was calculated measuring the conductivity of the whole material inside the chamber, such as both the vegetable and the KCl solution. The measurements of electrical conductivity were taken no later than 1 minute after each PEF treatment. The CDI was calculated as follow:

$$CDI = \frac{\left(\frac{\sigma_{u,high}}{\sigma_{t,high}}\right)(\sigma_{t,low} - \sigma_{u,low})}{(\sigma_{u,high} - \sigma_{u,low})} \qquad (4)$$

where, CDI is the disintegration index, σ (S / m) is the measured electrical conductivity, the subscripts *u* and *t* refer to the conductivities of untreated and PEF treated sample respectively and the subscripts *low* and *high* refer to low and high frequencies (0.5 kHz and 20 MHz). The values of electrical conductivity of the material inside the chamber (roots and KCl solution) were measured before and after each PEF treatment by connecting the treatment chamber to a

signal generator Rigol DG 1022 (Beijing, China) which provides a 4 V peak-to-peak sinusoidal signal of 0.5 kHz and 20 MHz and recording the voltage drop after the wave passed through the sample by using an oscilloscope TDS 2012 (Tektronix). To take out the parasitic capacitance of the system that becomes important at high frequencies a calibration curve using know resistors was done at the frequency of 20 MHz.

4.2.7. PEF pre-treatment before drying

Samples preparation before and after the PEF treatment

For the each PEF pre-treatment before drying, two whole parsnip roots (about 200 g of parsnip) and two whole carrots (almost 200 g of carrots) were used. After PEF treatment – samples were sliced as slabs (2 x 25 x 25 mm) before undergoing to drying process. Control samples did not undergo PEF treatment and - sliced into the same slab shape of 2 x 25 x 25 mm - underwent immediately to drying process (Figure 9.).

4.2.8. PEF unit and PEF treatment

The lab scale (PEF) unit previously described was used. The system employed a bipolar rectangular-shaped pulses having pulse width of 20 μs and a repetition rate of 50 Hz. The system was connected to a digital oscilloscope (Model No. TDS 2012, Tektronix, Beaverton, OR, USA) (Figure 10.).

The treatment module consisted of a batch chamber with 8 cm electrode gap and 50 cm^2 area for each electrode, resulting in a 400 cm^3 total volume. For each PEF treatment, two roots per time were placed on the Teflon base of the chamber and 195 g of KCl salt solution (0.156 g/L) was added to guarantee uniform distribution of the electric field. The output voltage of the machine was setup at 46% which gives an electric field strength of 0.9 kV/cm recorded in the result section of the machine after 1000 and 10000 pulses for carrots and parsnips respectively.

After that immediately, slicing and tray drying was done. Samples were processed at room temperature and were subjected to PEF treatments characterised by a total specific energy (Q) of 1.86 kJ/kg of carrot. It was calculated according to (Zhang *et al.*, 1995) based on:

$$Q = \frac{VAt}{m} \qquad (5)$$

Where, V is the voltage (kV), A is the current (A), t is the treatment time (s) and m is the sample mass (kg). After the treatment, samples were taken from the PEF chamber, drained with paper. After that immediately, slicing and tray drying was done.

4.2.9. Convective hot air drying

Convective drying was carried out at temperature of 50, 60 and 70°C, with air speed of 1 m/s in laboratory tray drier (Armfield Limited, Ringwood, Hampshire, UK), in order to reduce the average moisture of parsnip and carrot samples to about 20% w/w (Figure 11.). The dryer was pre-heated to temperature set point and then was loaded with 150 g of samples (in turn, slabs of parsnip and carrot) spread on holed plate in a single layer. The mass of the material was measured continuously by using a digital balance, located on the top of the oven for on line measurement of mass. The drying process were conducted in triplicate.

Determination of drying rate

The overall drying rate (Kar et al., 2003) was calculated using the following equation:

$$DR = \frac{M_o - M_t}{t} \qquad (6)$$

where, *DR* is overall drying rate (g water/g dry solid min^{-1}), M_0 is moisture content of at time 0 (g water/g dry solid) and M_t is moisture content of carrot and parsnip at time *t* (g water/g dry solid).

The moisture ratio (MR) of samples was calculated using the equation:

$$MR = \frac{M_t}{M_0} \qquad (7)$$

where, M_0 is moisture content at time 0 (g) and M_t is moisture content of carrot and parsnip at time t (g).

Measurement of drying temperature

Four K-type thermocouples connected to a data logger (Mod. 8856, Eurolec Instrumentation Ltd, Dundalk, Ireland) where used to measure the temperature of drying air along the convective dryer, two at the entrance and two at the outlet of the drying cavity.

4.2.10. Quality analysis of carrot and parsnip

Moisture content

The moisture content was determined according to oven drying method at 105 °C until a constant weight reached (AACC, 2000).

Color analysis

Color was obtained through a colorimeter Minolta Chroma Meter II Reflectance CR-400 (triple flash mode aperture 10 mm).

Texture analysis

An Instron Universal Testing machine (Mod. 5544, Instron Corporation, high Wycombe, UK) equipped with a 500 N load cell was fitted with a Warner Bratzler flat blade (1.0 mm thick). The test was performed at the speed of 50 mm/min. Texture was assessed by Warner–Bratzler shear force measurements, which indicates the maximum shear force (Newtons) required to cut the sample. Data were analysed by Bluehill 2 software package (Version 2.5, Instron Corporation, High Wycombe, UK). The maximum compression load values (N) of the dried carrots and parsnips were recorded using the method described by (Zell *et al.*, 2010).

Carotenoid analysis of carrot

Extraction and determination

Carotenoids were extracted according to the method reported by (Panfili, Fratianni, and Irano, 2004) and (Fratianni *et al.*, 2013b, 2015) with slight modifications: 0.5 g of freeze dried carrots was saponified under nitrogen in a screw capped tube by adding 5 mL of ethanolic pyrogallol (60 g/L) as antioxidant, 3 mL of ethanol (95%), 1 mL of sodium chloride (10 g/L), and 2 mL of potassium hydroxide (600 g/L). The tubes were placed in a 70°C water bath and mixed every 5–10 min during saponification. After alkaline digestion at 70°C for 45 min, the tubes were cooled in an ice bath and 15mL of sodium chloride (10 g/L) were added. The suspension was then extracted with 15 mL portions of n-hexane/ ethyl acetate (9:1, v/v) until it became colorless. Recovery tests made on samples and pure standards showed that the used saponification procedure did not cause isomerization/degradation of the investigated carotenoids (Fratianni et al., 2010; Panfili *et al.*, 2004). The organic layers, containing carotenoids, were collected and evaporated to dryness; 146 the dry residue was dissolved in methanol: MTBE (50:50 v/v) and an aliquot of the carotenoid extract (25 µl) was separated, as in (Mouly, Gaydou, and Corsetti 1999), by a reverse-phase HPLC system. A HPLC Dionex (Sunnyvale, CA) analytical system consisting of a P680 solvent delivery system and a 25 µl injector loop (Rheodyne, Cotati) was used. Separation was performed by a YMC (Hampsted, NC, USA) stainless steel column (250×4.6 mm i.d.), packed with 5 µm silica spheres that were chemically bonded withC30 material, at a flow rate of 1 mL/min. The mobile phase was methanol: MTBE: water (v/v/v). The eluted compounds were monitored by a photo-diode array detector (Dionex, Sunnyvale) set at 430 nm.Data were stored and processed by a Dionex Chromeleon Version 6.6 chromatography system (Sunnyvale, CA).

Identification and quantification

β-carotene, α-carotene, Phytoene,13-cis-β-carotene and9-cis-β-carotenewere identified on the basis of diode array spectral characteristics, retention times, and relative elution order, compared with known commercially available standards. α-carotene, 9-cis-β- carotene, and 13-cis-β-carotene standards were purchased from CaroteNature (Lupsingen, Switzerland); all-trans-β-carotene was from Sigma Chemicals (St. Luis, MO, USA). Purity for all standards was above 95% (as certified by the suppliers). All carotenoid standards were spectrophotometrically quantified and diluted in methanol: acetone (2:1, v/v) to give a final concentration of 25 µg/ml. The concentration range was 5– 25 µg/ml for every carotenoid standard. Identified carotenoids were quantified using calibration curves of respective standard solutions. Total carotenoids were expressed as a sum of single quantified carotenoids.

Feurosine content

Furosine content was determined by HPLC, according to (Resmini, Pellegrino, and Battelli, 1990). A sample amount, corresponding to about 30-70 mg of protein, was hydrolyzed under nitrogen with 8 ml of 8 N HCl at 105 °C for 23 h. Afterwards, the hydrolyzate (0.5 ml) was purified on a Sep-Pak C18 cartridge (Waters), diluted and analyzed by HPLC (Waters), equipped with an Alltech furosine dedicated column (250 x 4.6 mm) (Alltech, Derfield, IL, USA). Furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France).

Total soluble phenolics (TSP) analysis

A procedure for TSP analysis was adapted from (Swain and Hillis, 1959) with slight modification. Frozen specialty crop samples were ground to powder using a Waring Laboratory Blender (Waring Commercial, Torrington, CT). Three replicates were used for extraction of phenolic compounds from each powder sample. From each extract, duplicate samples were used for final analysis. After 2–10 g of powder samples were added into a 50 mL centrifuge

tube, 20 mL HPLC grade methanol was added. Tubes were capped, vortexed for 15 s, and then stored at 4°C overnight. The resulting homogenates were vortexed for 15 s, and then clarified by centrifugation (29,000 × g, 15 min at 4°C) using a SORVALL RC 5C Plus centrifuge (Kendro Laboratory Products, Newtown, CT). A total of 150 L methanol extracts were taken from the clear supernatant and then diluted with 2400 L nanopure water, followed by 150 L of 0.125 mol L−1 Folin-Ciocalteu reagents and incubated for 3 min at room temperature. The reaction was stopped by adding 300 L of 0.5 mol L−1 Na_2CO_3 and the mixture was incubated for 25 min. Absorbance readings at 725 nm were taken using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). A blank prepared with methanol was used as control. The level of TSP for each sample was determined by using a standard curve (0–0.375 mg mL^{-1}) developed with chlorogenic acid and expressed as chlorogenic acid equivalent (CAE) mg per kg of fresh weight (mg kg^{-1}).

4.2.11. Microscopic images

Microscopic analyses were carried out on three thin sections of carrot and parsnip, both fresh and dehydrated. The samples were treated with Lugol liquid to highlight the starch and with toluidine blue to highlight the lignified parts. Each section was then placed in a glass slide with water and covered with a cover slip. For observation was used the optical microscope Nikon YS100 using magnification x 10 and x 40.

4.2.12. Estimation of effective diffusivity in the processed samples

In order to estimate the effective diffusivity of moisture in the processed samples, the mass transport phenomenon has been considered being purely molecular, described by the Fick's second law:

$$\frac{\partial M_t}{\partial t} = D_{eff} \frac{\partial^2 M_t}{\partial z^2} \quad (8)$$

Under a number of hypothesis (diffusivity being constant and independent by the moisture content, shrinkage being ignored and isothermal conditions), the following analytical solution applied to thin materials undergoing drying (Wiktor *et al.*, 2016):

$$MR = \frac{8}{\pi^2} exp\left(-\frac{D_{eff}\,\pi^2\,t}{4\,L^2}\right) \quad (9)$$

Where, D_{eff} is the effective diffusivity ($m^2\ s^{-1}$), t is the time and L is the half-thickness of the slices in turn of carrots and parsnips.

Using eq. (9), diffusivity can be calculated once *MR* is measured during the processing time, *t*, after the lag time.

CHAPTER V

5. RESULTS AND DISCUSSION

5.1. Results and discussions (goji berry)

5.1.1. Proximate composition analysis

The nutritional composition of fresh and dried goji berries is shown in (Table 4) Fresh goji berries have 77.4 % moisture, 1.1 % fats, 2.0 % proteins, 15.3 % carbohydrates and 2.9 % fibre. In dried goji berries 4.4 % fats, 10.2% proteins, 61.3 % carbohydrates and 11.4 % fibre were found. Similar results on dried goji were reported by (Endes *et. al.*, 2015). Our data suggest that dried fruits contain notable levels of dietary fibre, either as water-soluble form (2.6 %) or as insoluble form (8.8 %). The ratio between insoluble and soluble fibre is about 3:1. Dietary fibre intake recommendation for adults is 25 g/day (Larn, 2014). With the consumption of a portion of 30 g of dried fruits, dietary fibre intake for the adults is about 14 % of its daily-recommended intake. Taking into account the European law (Regulation CE 1924/2006), dried goji can be declared in label with the claim "high fibre content", since it contains at least 6 g of fibre per 100 g. Finally, fresh and dried goji berries provide about 87 and 348 kcal/100 g, respectively.

5.1.2. Quality analysis

Mineral composition

The content of both macro and microelements in goji berries is reported in (Table 5.) Potassium (K) is the predominant element (276.2 mg/100 g and 881.9 mg/100 g for fresh and dried fruits, respectively), followed by sodium (Na). Potassium and sodium play an important role in regulating blood pressure and the body's acid-base balance (Clausen *et al.*, 2013; Siddhuraju *et al.*, 2001). Goji could also be a good source of phosphorus (P) and calcium (Ca), with an

appreciable concentration of magnesium (Mg), which is needed to prevent heart disease and growth retardation. A discrete amount of copper (Cu), iron (Fe) and manganese (Mn) were also found (Bellaio *et al.*, 2016. Endes *et al.*, 2015 and Llorent-Martínez *et al.*, 2013) reported slightly different results. As in any other plant food, the mineral content of berries reflects the soil in which they are grown. It is important to highlight that essential and nonessential element concentration is dependent on the soil characteristics, the physiology of the plant, the water source composition, and fertilizers, insecticides, pesticides, and fungicides used in the plantations. Plants can absorb, carry, and accumulate chemical elements. Each species has its own requirements and differing levels of tolerance when absorbing and accumulating an element. The movement of the inorganic constituents is selectively controlled by the plant, with some being easily absorbed and others impeded to a different degree (Naozuka *et al.*, 2011).

(Table 6) reports the percentage contribution to the RDA of 100 g of fresh and dried goji berries, according to (Reg. EU 1169/2011). For dried goji berries, the percentage of RDA per portion (30 g) is also reported. From data, fresh goji berries can be declared on the label as a source of Cu; in fact, 100 g of fresh goji berries contributed to about 25% of the RDA. The contribution of other minerals from fresh goji is low. Dried goji berries can be declared as source of K, P, Cu, Fe Mn, and Zn. A consumption of 30 g of dried goji per day contributes to the RDA approximately of 25 % for Cu, 13 % for K and less than 10 % for other elements.

Carotenoid, tocolpherol and ascorbic acid amounts

Lycium fruit is considered to be a good food source of zeaxanthin. Zeaxanthin is a yellow pigment, an isomer of lutein and a derivative of β-carotene. When ingested, zeaxanthin accumulates in fatty tissues, but especially in the macula, a region of the retina. It has been reported that this compound may help to protect the macula from degeneration, which can be induced by excessive sun exposure (UV light) and by other oxidative processes (Rosenthal *et*

al., 2006; Trieschmann *et al.*, 2007). The reddish-orange color of L. barbarum fruits is derived from a group of carotenoids, which make up only 0.03–0.5% of the dried fruit (Peng *et al.*, 2005).

(Table 7) shows HPLC carotenoid analysis of fresh and dried fruits. Unsaponified carotenoids, determined after solvent extraction, and saponified carotenoids, determined after saponification of the extract, are reported. Unsaponified carotenoids are characterized by one significant peak, identified as zeaxanthin dipalmitate, the dominant ester of goji berries (Inbaraj *et al.*, 2008). Beta-carotene, and, before zeaxanthin dipalmitate peak, other unidentified peaks, probably carotenoid esters (Inbaraj *et al.*, 2008), are also present. The amount of zeaxanthin dipalmitate in dried fruits is about 158.8 mg/100 g and of β-carotene is about 1.0 mg/100 g. (Inbaraj *et al.*, 2008), found values of zeaxanthin dipalmitate and of β-carotene of 114.3 mg/100 g and 2.4 mg/100 g, respectively. Saponification of the extract is necessary to convert esters to free-compounds and it is often used to remove chlorophylls, lipids and other analytical interferences (Fratianni *et al.*, 2015). The saponified extract of dried fruits shows high zeaxanthin contents (about 190 mg/100 g) and small lutein and β-cryptoxanthin amounts (about 6 mg/100). Small amounts of lutein were also found, after saponification, in a work of (Zhao *et al.*, 2013). As a dietary supplement for eye health (Cheng *et al.*, 2005), a dose of 15 g per day was deemed beneficial in supplying adequate zeaxanthin (estimated at 3 mg/day). Thirty g of our goji samples provide a zeaxanthin amount of 14 mg/die (fresh fruit) and 48 mg/die (dried fruit).

(Table 8) shows the tocol amounts in goji berries. Goji berries were found as a source of α- and β-tocopherol (about 1.4 and 1.0 mg/100 g, respectively, in fresh fruits, and 5.5 and 4.2 mg/100 g, respectively, in dried fruits). (Table 8) also reports values of vitamin E activity provided by 100 g of product, expressed as Tocopherol Equivalent (TE) (mg/100 g product)

(Sheppard *et al.*, 1993). Taking into account the Recommended Daily Allowance (RDA) for vitamin E, which is of 12 mg/die (Regulation EU 1169/2011), 100 g of fresh goji berries contribute approximately 16 % of the RDA, while 100 g of dried fruits contribute approximately 66 % of the RDA, so that to be declared in label as a source of vitamin E. A portion of dried goji berries (30 g) contributes approximately 20 % of the RDA. The concentration of vitamin C was about 40 mg/100 g in fresh fruits and 38 mg/100 g in dried fruits. (Donno *et al.*, 2015), report an amount of about 42 mg/100 g in dried goji berries. Taking into account the Recommended Daily Allowance (RDA) for vitamin C of 80 mg/die (Regulation EU 1169/2011), 100 g of fresh or dried goji berries contribute approximately 50 % of the RDA, so that they can be declared on the label as a source of vitamin C. A portion of dried goji berries (30 g) contributes about 16 % of the RDA.

Color evaluation

The quality of fresh, before and after pretreatment, and dried samples was evaluated through measurements of color and the results were presented in (Table 9).

The pretreatment did not determine a change in the color of fresh samples. As a result of drying, the variable L*, a* and b* decreased in both samples, even if a lower decrease in Hue angle and of Chroma was observed in TR-Abr samples than for UTR ones. These results confirmed the effectiveness of abrasive pretreatment in better color preservation of dried goji berries.

Antioxidant activity evaluation

The antioxidant activity of dried goji berries was found significantly higher (20% inhibition of DPPH/mg db) than other fruits, such as apricot (10% inhibition of DPPH/mg db) and Red Globe grape (1% inhibition of DPPH/mg db) as reported by (Albanese *et al.*, 2013) and (Adiletta *et al.*, 2015), respectively. In details, the antioxidant capacity of TR-Abr dried

samples was stronger than UTR dried ones (Figure. 12). Maillard reaction develop some new compounds that show antioxidant activity. Carotenoid will remain same.

Sugar evaluation

Goji berries are a rich source of carbohydrate. In this study, glucose, fructose and trehalose were detected. As reported elsewhere for grapes (Carranza-Concha *et al.*, 2012) and plums (Cinquanta *et al.*, 2002), a reduction of sugar content was observed during drying, probably caused by non-enzymatic browning reactions (Figure 13). In details, the main sugar were fructose and glucose, which amount in the fresh goji were 90.13 mg/g db and 80.34 mg/g db respectively. Those were reduced to 72.55 mg/g db, 64.87 mg/g db and 67.68 mg/g db, 57.64 mg/g db in case of UTR and TR respectively after drying but in case of trehalose, no relevant differences were observed. This behaviour was probably justified by higher drying time required for untreated samples to reach the set moisture content.

5.3. Drying kinetics

To compare the effect of pretreatment on the drying kinetics of goji berries, the curves of M_t/M_0 versus drying time were shown in (Figure 14). It can be seen that M_t/M_0 of goji samples decreased with the increase of drying time and the abrasive pretreatment shortened the drying time significantly compared to the untreated samples. In particular, the TR-Abr samples showed faster moisture loss with respect to UTR ones: the drying time decreases from 21h for UTR samples to 15 h for TR-Abr samples to reach the final M_t/M_0 value of 0.012.

5.2. Results and discussion (carrot and parsnip)

5.2.1. Proximate composition

The nutritional composition of fresh and dried carrot & parsnip is shown in (Table 10) Fresh carrot and parsnip have, expressed as g/100 g, 85.3 % moisture, 0.3 % fats, 0.7 % proteins, 7.6 % carbohydrates and 3.7 % total fibre was found in fresh carrot. On the other hand, 81.5 % moisture, 0.4 % fats, 2.3 % proteins, 9.6 % carbohydrates and 4.3 % total fibre was found in fresh parsnip. In dried goji berries 4.4 % fats, 10.2% proteins, 61.3 % carbohydrates and 11.4 % fibre were found.

5.2.2. Cell disintegration Index

The application of PEF results in the electroporation, which depends on the perforation of the cell membrane. The leakage of intercellular content leads to the change of the tissue electrical properties. Therefore, electroporation efficiency can be assessed by the means of electrical conductivity (EC) measurement (Ade-Omowaye *et al.*, 2003a). The character of electroporation is directly connected with material properties, including cell's size and root physical organization. The anatomy of fresh carrots is known as bi-dimensional anisotropic, due to cells elongated along the direction of the growth: mainly, an outer region is identified in the cortex, while the stele constitutes the inner vascular tissue (Figure 26a and 26b). It was found out that anisotropy to a certain extent remains after thermal treatments and drying (Voda et al., 2012). Parsnip cells are relatively rounded and small with a diameter about 100 µm, similar to carrot cells (Bengtsson and Tornberg, 2011). Parsnip root cells accumulated more starch via enlargement of starch granules; this observation is due to the growing starch granules; this observation is due to the growing starch granules occupied more and more of the cell volume (Figure 26c and 26d). The vacuoles of parsnip did not maintain their relative size during growth, since the growing starch granules occupied more and more of the cell volume.

The cell disintegration index (CDI) has been used to analyse the degree of cell disruption owing to the PEF pre-treatment. In our trials carrots showed a sharp increase of the index, followed by a plateau corresponding to a CDI stable value of about 0.8, which denoted a significant degree of damaged tissue (Figure 15), after about 100 msec of treatment and 1000 pulses (Table 2). Otherwise parsnips tissue were almost completely destroyed after 200 msec treatment time and 10000 pulses (Table 3) as it reached the CDI value equal to about 1 (Figure 16).

Such data indicated that carrot tissue was more abundant in substances able to conduct a current, for instance ions of dissociated salts, in less time and drastic conditions. The different structures of the material can contribute to the reduction of the electroporation effectiveness, which in this study was expressed by the different CDI values after PEF pre-treatment in the case of parsnip tissue. As mentioned before, the difficulty to break and disintegrate parsnip cell walls could be related to its relatively high content of insoluble fiber. In particular, the total Klason lignin was 1.9% in parsnip originated from lignified tissues, whereas in carrot was less 0.9% (Grigelmo and Belloso, 1998; Theander *et al*., 1995).

5.2.3. Drying kinetics

Evolution of moisture ratio (MR) versus the processing time for both untreated and PEF pre-treated samples of carrots and parsnips, undergoing drying at 50°C, 60°C and 70°C, are shown in (Figure 17) and (Figure 18), respectively. Drying time of the PEF pre-treated carrot reduced up to 8% and 21% at 50°C and 60°C but there was no effect on drying time in case of 70°C in comparison to conventional drying (Table 11).

PEF pre-treatment reduced drying time of parsnip up to 6%, 3% and 24% at 50°C, 60°C and 70°C.The response tissues following PEF treatment in relation to different diffusion properties during drying is complex and can be attributed to the differences in morphology, i.e. different fiber content and porosity of the tissues.

5.2.4. Quality analysis

Color evaluation

The results indicated that PEF pre-treatment did affect the color of dried carrots. The PEF pre-treated dried carrots were characterized by lower value of *L** compared to untreated dried ones (Table 12).

According to (Grimi *et al.*, 2010), it might be linked with the electroporation phenomenon and release of intracellular content (e.g. enzymes and the substrates for enzymes, among others), which take place during PEF treatment. A significant increase in *b** values about PEF pre-treated parsnip's samples could be explained by an initial stage of Maillard's reaction (Table 13).

Texture analysis

The PEF pretreatment did not affect apparently the shear stress needed to cut the samples, both for carrots and parsnips, after drying at 50 and 60°C, as shown in (Figure 19) and (Figure 20). Despite of certain loss in the firmness of tissue structure, induced by the PEF, the carrot tissue remained rather rigid after the PEF treatment (Figure 19). It can be concluded that the loss of turgor, induced by PEF, did not affected the texture properties of carrot and, especially, of parsnip, where starch is the major component of the dry matter (Lebovka *et al.*, 2004).

Different is the dried vegetables behavior at 70°C, where the combined effect of the PEF and high temperatures causes a significant increase of the force required to cut the sample, especially in the case of parsnip, due to the higher starch content.

Carotenoid amounts

5 different carotenoid compounds were found in carrots. The major identified carotenoids were: (i) β-carotene (ii) α-carotene and (iii) Phytoene peaks. Other major peaks which were

tentatively identified named 13cis β-carotene and 9cis β-carotene. PEF facilitates the oxidation and enzymatic reaction this causing carotenoid reduction (Table 14, 15, 16, 17).

Furosine content

The convective treatment at 50°C does not induce significant changes in the parsnips. The development of the Maillard reaction is not significant and the furosine values are kept low (11.1 mg / 100g of protein) and similar to those found in the parsnip lyophilized (Figure 21). However, furosine in PEF samples at 70°C the content of furosine does not increase, is reduced. This means that the Maillard reaction almost certainly is evolving towards the secondary phase. Given the composition of the stingray, the most suitable indicator to be used would be the hydroxymethylfurfural (HMF).

Total soluble phenolic (TSP) analysis

PEF treated parsnip showed a significant but slight increase in TSP (Figure 23) compared to conventional on the other hand, carrot showed completely opposite behaviour (Figure 22), according to the results reported elsewhere (Piga *et al*., 2003). Although different tissue structure, variety, maturity stage, and other differences may be contributing factors regarding this. The PEF treatment did not affect the texture properties of carrot and parsnip at 60 and 70 °C, whilst at 70°C a significant increase of the force required to cut the slices, was detected. Texture measurements of the rehydrated samples revealed that PEF determined the softening of the tissue due to the loss of turgor caused by the permeabilization.

5.2.5. Effective moisture diffusivity

The analysis of moisture ratio decay during processing time, allowed to estimate the value of moisture diffusivity, according with eq. (9), which are reported in (Table 18).

In case of untreated sample drying, going from 50 to 70°C, diffusivity values were found in a range between $8.74 \cdot 10^{-11}$ to $1.41 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for carrots and between $7.17 \cdot 10^{-11}$ to $1.03 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for parsnips, respectively. As expected, the effective moisture diffusivity increased with the drying temperature. Effective water diffusion coefficient of the PEF pre-treated samples, going from 50 to 70°C, ranged between $9.67 \cdot 10^{-11}$ to $1.40 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for carrots and between $7.41 \cdot 10^{-11}$ to $1.33 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for parsnips, respectively.

Comparing the diffusivity values obtained for untreated carrot's samples with respect to those related to PEF pre-treated samples, it is possible to observe that PEF pre-treatment on carrots, dried at 50° and 60°C, led to an increase of diffusivity respectively of 11% and 22%. In contrast, for drying operating at 70°C, the PEF pre-treated samples shown a diffusivity slightly lower than the value related to untreated sample drying at same temperature. On the other hand, it was already stated, in the discussion about the drying times, that the impact of PEF pre-treatment on carrots did not enhance drying at 70°C.

Previous works (Amami *et al.*, 2008; Viktor *et al.*, 2016) reported higher estimated values of moisture diffusivity in carrots undergoing drying after PEF pre-treatment, probably owing to the different conditions adopted.

5.2.6. Energy consumption during drying

Energy consumption was measured through power meter during drying experiments and measurements were presented in (Figure 24 and Figure 25).

Drying time reduction can result in a decrease in energy consumption (Wiktor *et al.*, 2013; Wu *et al.*, 2011). The shortest drying time was observed at 60°C for the PEF treated carrots (Figure 24) and 70°C for PEF treated parsnip (Figure 25).

As a result, low power required for PEF treated carrot at 60°C (3.31 kW-h) compared to 50°C and 70°C (4.82 and 3.59 kW-h) on the other hand, for PEF treated parsnip at 70°C (3.27 kW-h) compared to 50°C and 60°C (4.95 and 3.58 kW-h) respectively.

CHAPTER VI

6. CONCLUSIONS

Goji berries cultivated in Italy were confirmed as an important source of healthy compounds, providing a significant contribution to the diet, in terms of some inorganic nutrients, and of dietary fibre, zeaxanthin, vitamins E and C. In particular, taking into account the Recommended Daily Allowance (RDA) for minerals and vitamins established by the Commission of the European Communities, dried goji berries can be declared as a source of K, P, Cu, Fe, Mn and Zn. Moreover, both fresh and dried berries can be declared on the label as a potential source of vitamins E and C.

The effect of abrasive pretreatment on drying kinetics of goji berry was studied during hot air drying process at 60°C. Moreover, the quality of goji berry before and after drying was analysed. Pretreated berries (TR-Abr) showed reduced drying times and lower color changes and similar sugar content than untreated samples (UTR). The faster release of moisture may be attributed to the lower resistance offered for diffusion of moisture by the skin, since wax present on the peel surface was removed by the physical pretreatment. Moreover, the antioxidant capacity of TR-Abr dried samples was found relative stronger than UTR dried. These dried samples could be used as a dietary source of natural antioxidants and be worthy of development and utilization. In future work, the antioxidants present in goji berry fruit will be identified and characterized.

On the other hand, the effect of Pulsed electric field was tested on carrot and parsnip slices. The cell disintegration index (CDI) during the PEF treatment in carrots raised a value of about 0.8, after about 100 msec of treatment and 1000 pulses, while parsnips tissue were almost completely destroyed after 200 msec treatment time and 10000 pulses. As expected, the

effective moisture diffusivity increased with the drying temperature, drying time of the PEF treated parsnip was reduced up 24% at 70°C and up to 21% at 60°C for carrot. PEF pre-treatment did affect the color of dried carrots, by reducing the value of brightness (L*) in dried carrots and increasing the yellow index (b*) in dries parsnip. The PEF pre-treatment affected the shear stress needed to cut the samples only after drying at 70°C. The higher retention of color in the case of PEF-treated material can be due to the higher shrinkage, thus reducing the porosity. This protected the material and reduced oxidation of the carotenoids. In parsnip samples differences were shown in color parameters owing to pre-treatments, in particular the L* values decreased with PEF pre-treatment, due to Maillard reaction.

The PEF treatment causes a non-thermal rupture of cellular membranes and decrease or loss of the turgor component of cells. Instrumental texture measurements indicated that there were no differences in the shear force (Newtons) within carrots and parsnip pretreated by PEF and not, after drying at 50 and 60 °C.

Different is the dried vegetables behavior at 70 ° C, where the combined effect of the PEF and high temperatures causes a significant increase of the force required to cut the sample, especially in the case of parsnip, due to the higher starch content.

5 different carotenoid compounds were found in carrots. The major identified carotenoids were: (i) β-carotene (ii) α-carotene and (iii) Phytoene peaks. Other major peaks which were tentatively identified named 13cis β-carotene and 9cis β-carotene.

The Convective treatment at 50 ° does not induce significant changes in the parsnips. The development of the Maillard reaction is not significant and the furosine values are kept low. However, furosine in PEF samples. At 70 ° C the content of furosine does not increase, is reduced. This means that the Maillard reaction almost certainly is evolving towards the secondary phase.

PEF could be an effective pretreatment on drying in terms of reduction of time and energy costs. However, the operative conditions should be compatible with possible deterioration in quality of the product to be dried.

CHAPTER VII

7. REFERENCES

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CHAPTER VIII

8. APENDIX

Figure 1. Goji plants from Sibari, Italy.

Figure 2. Parsnips and carrots.

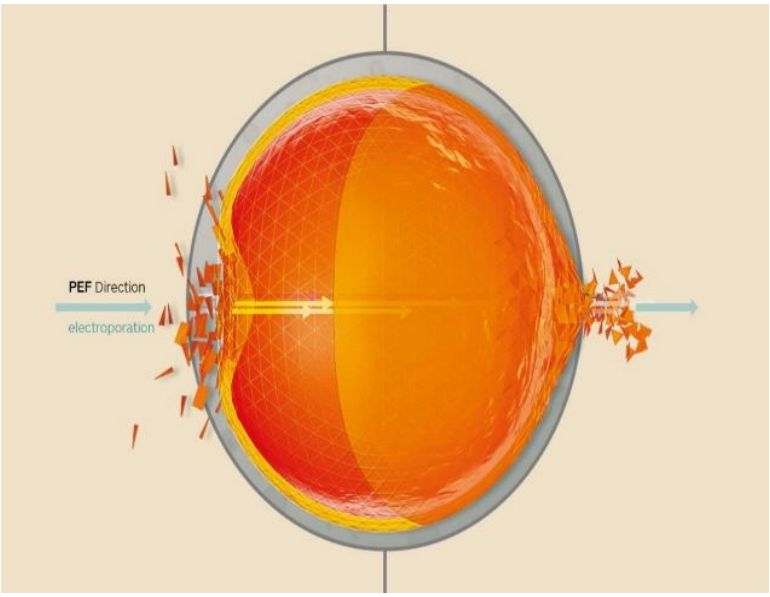


Figure 3. Cell cytoplasm is surrounded by a thin semi-permeable layer, the cell membrane.

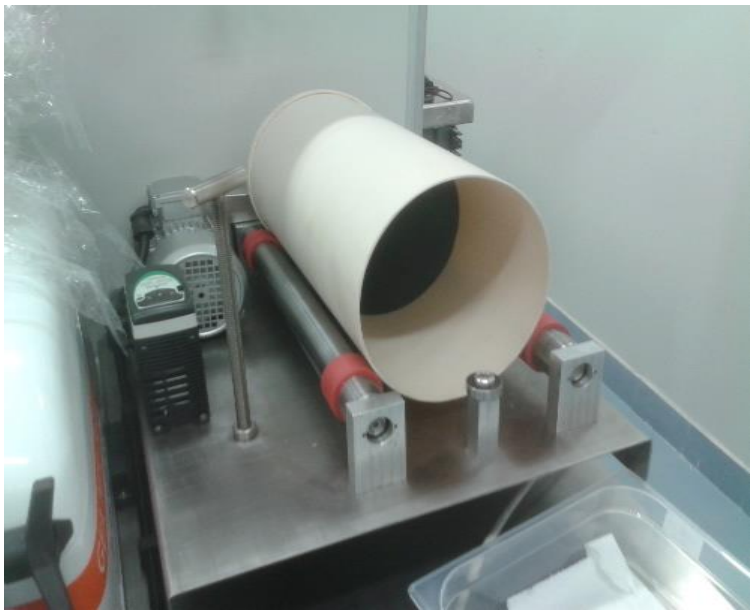


Figure 4. Pilot system with motorized drum for abrasive treatment.

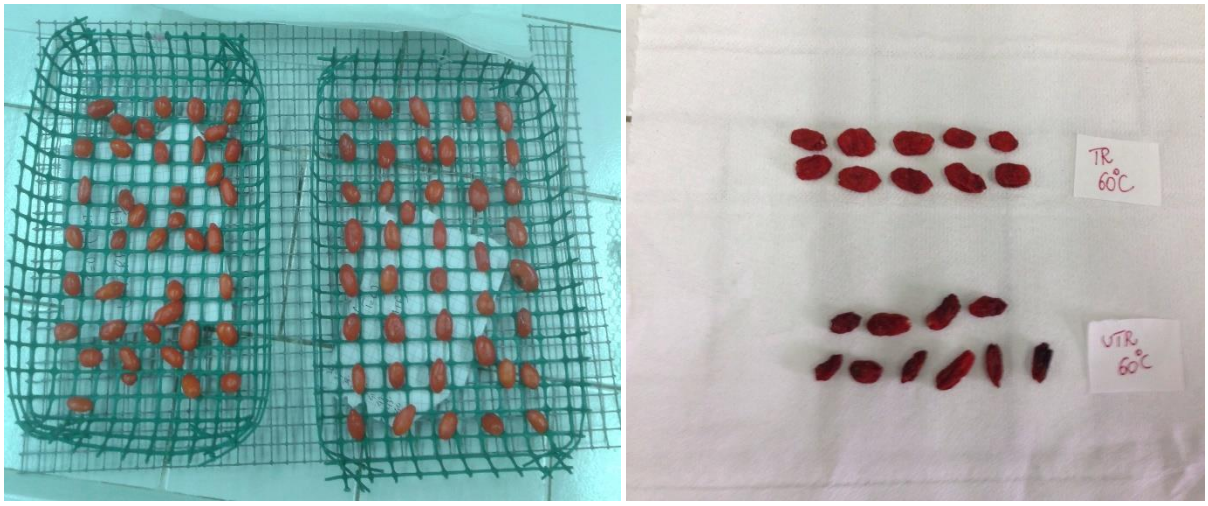


Figure 5. Effect of waxy layer on fruits after abrasive treatment and drying.

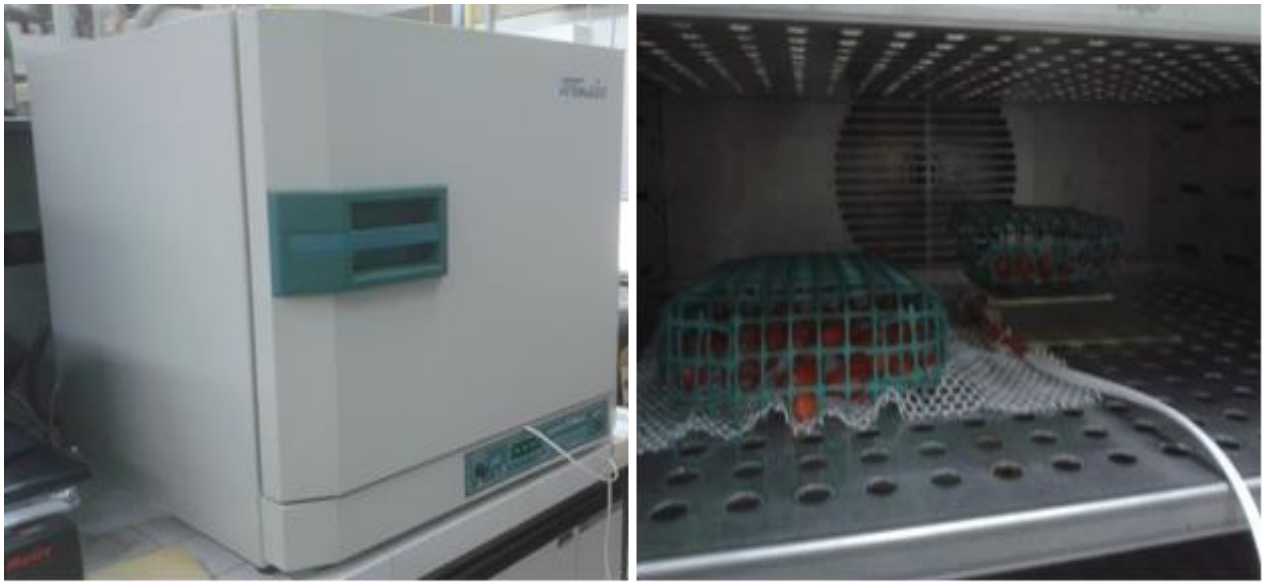


Figure 6. Convective hot air dryer.

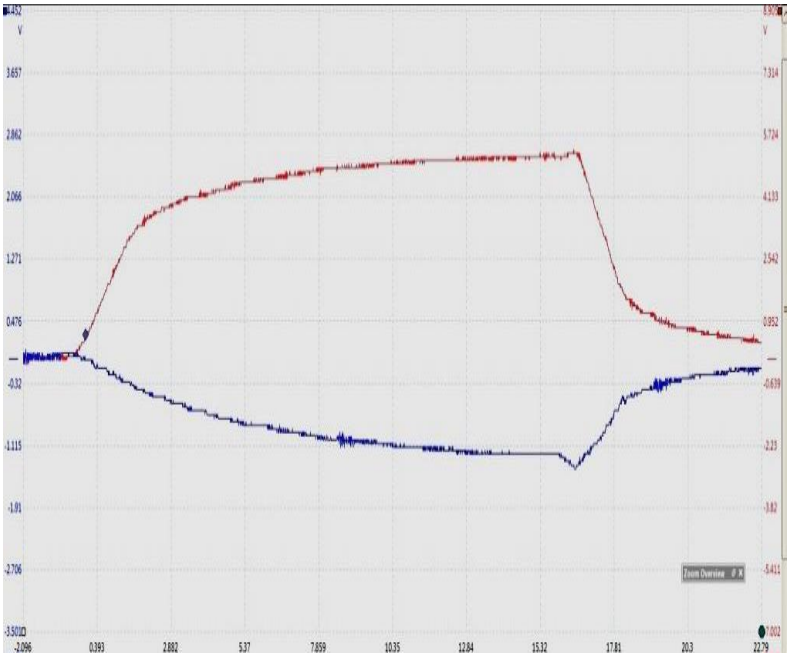


Figure 7. Pulse shape.

Figure 8. Sample preparation for measuring the cell disintegration index.

Figure 9. Sample preparation for PEF treatment.

Figure 10. Lab scale PEF unit.

Figure 11. Conventional tray drier.

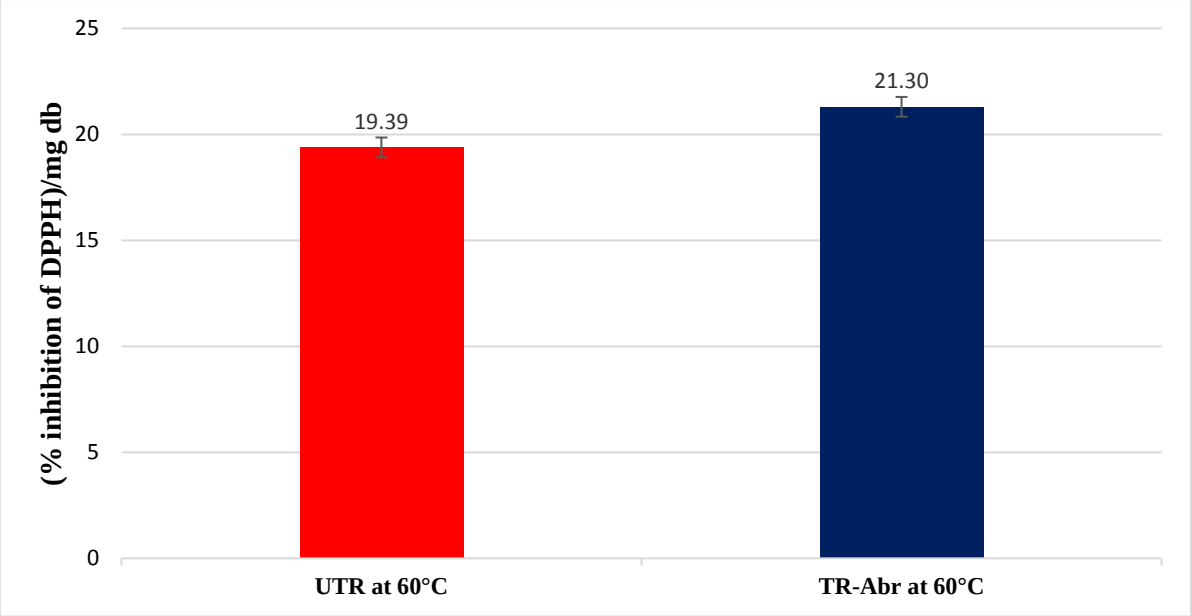


Figure 12. Antioxidant activity of untreated (UTR) and pretreated (TR-Abr) dried 60°C samples.

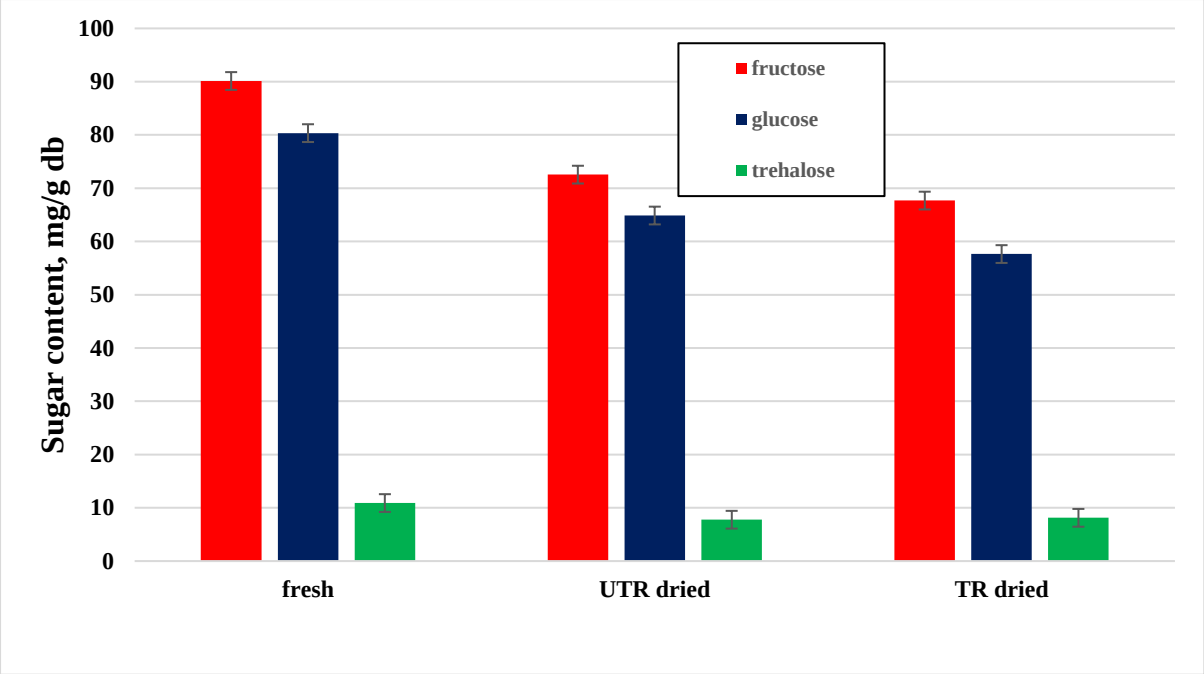


Figure 13. Sugar content (mg/g db) for fresh and dried samples.

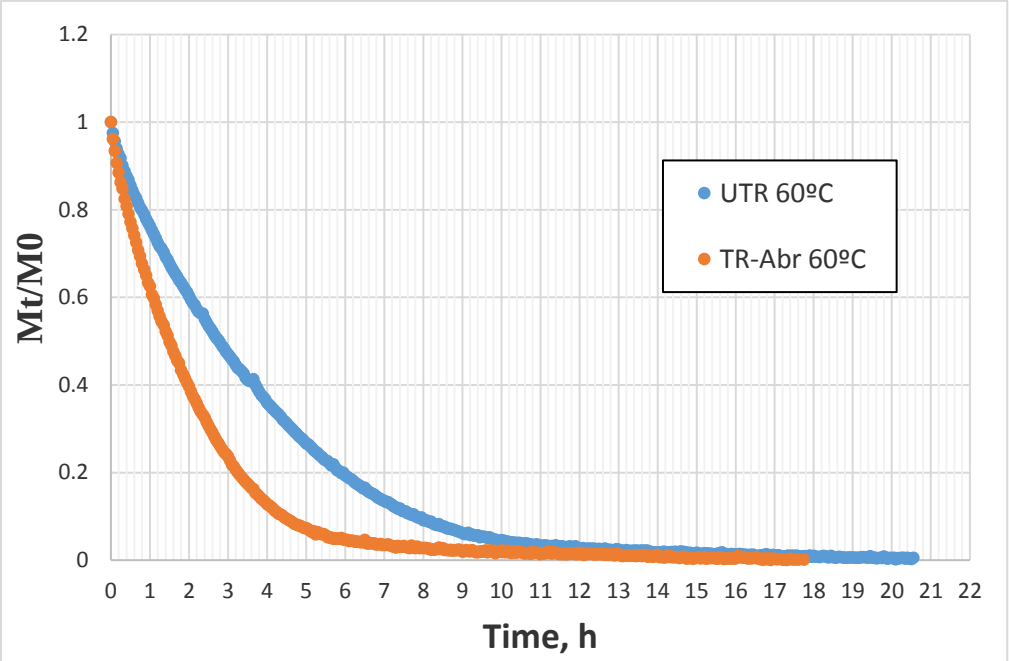


Figure 14. Moisture ratio of untreated (UTR) and pretreated (TR-Abr) samples during drying at 60°C.

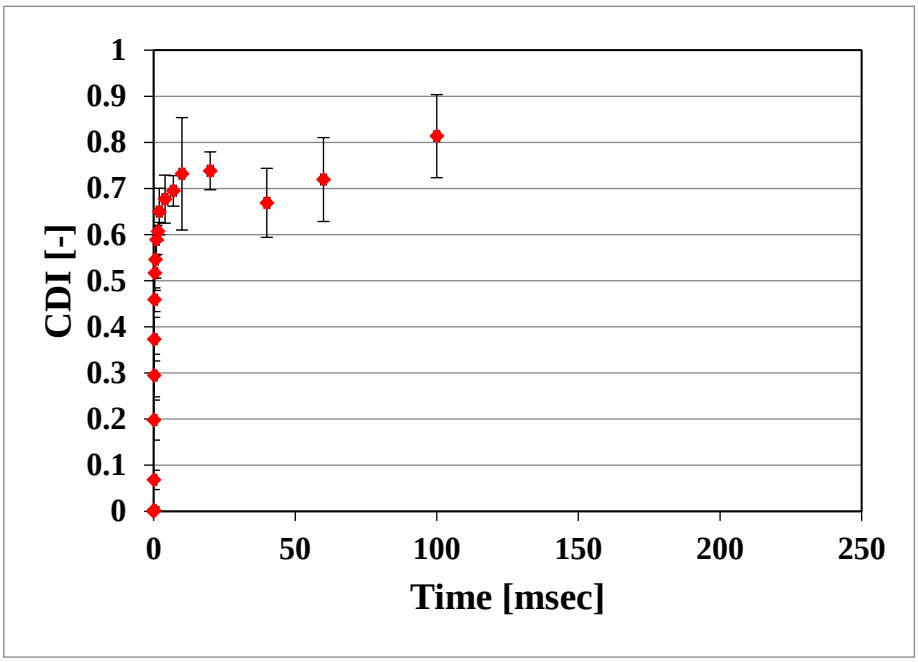


Figure 15. Cell disintegration index in carrot.

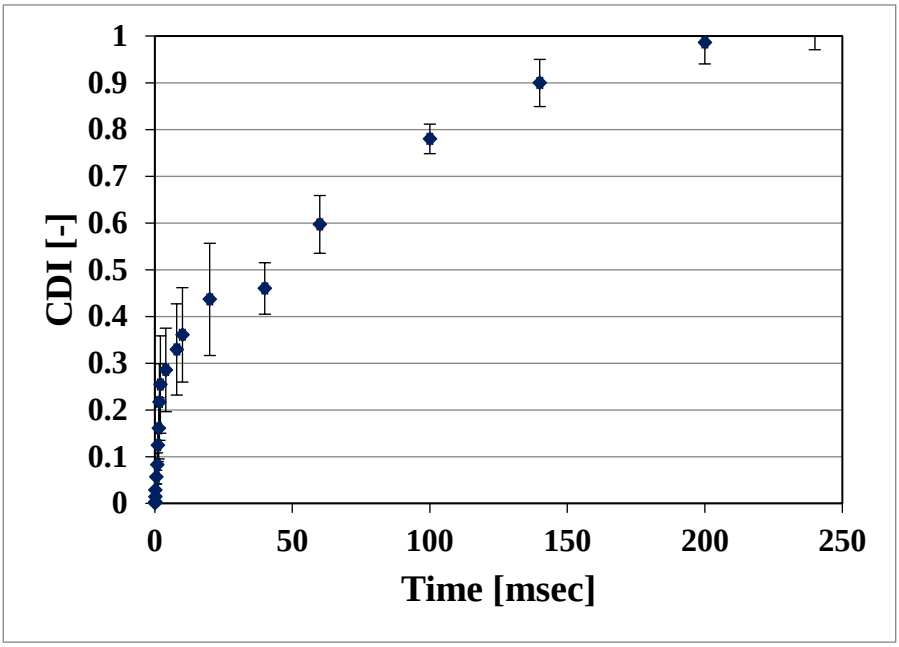


Figure 16. Cell disintegration index in parsnip.

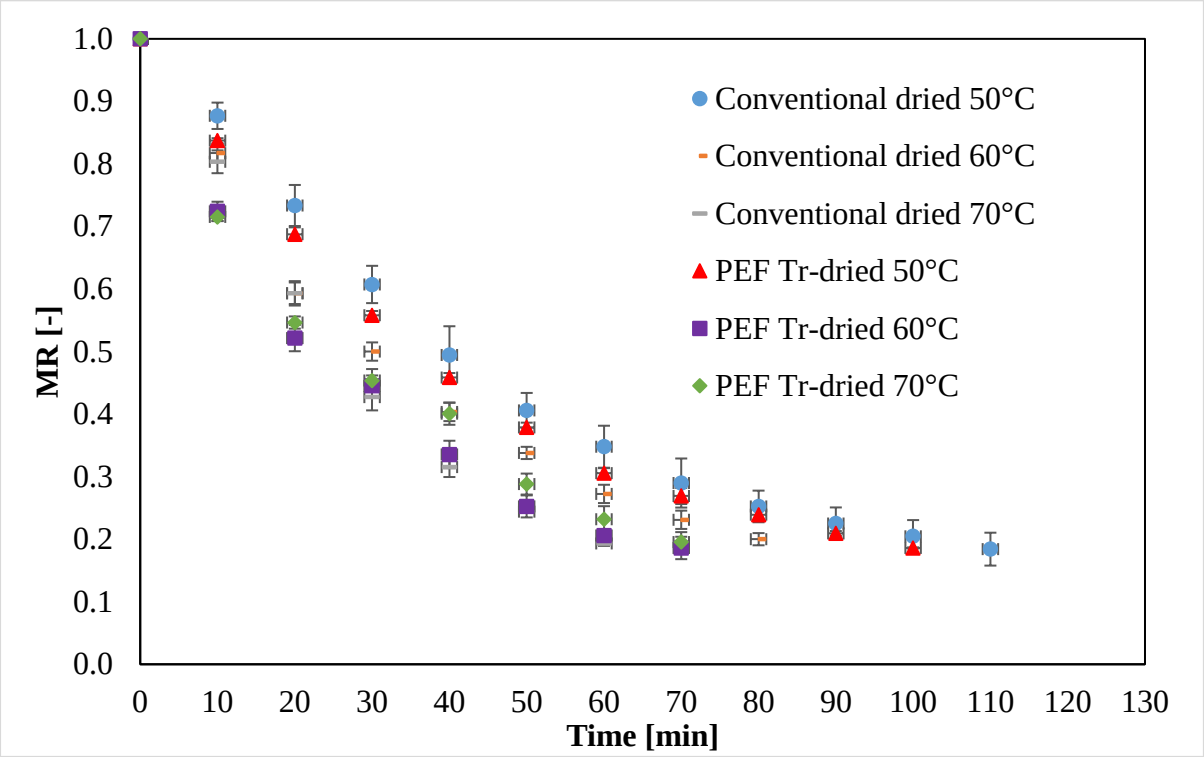


Figure 17. Drying kinetics of conventional and PEF treated carrots.

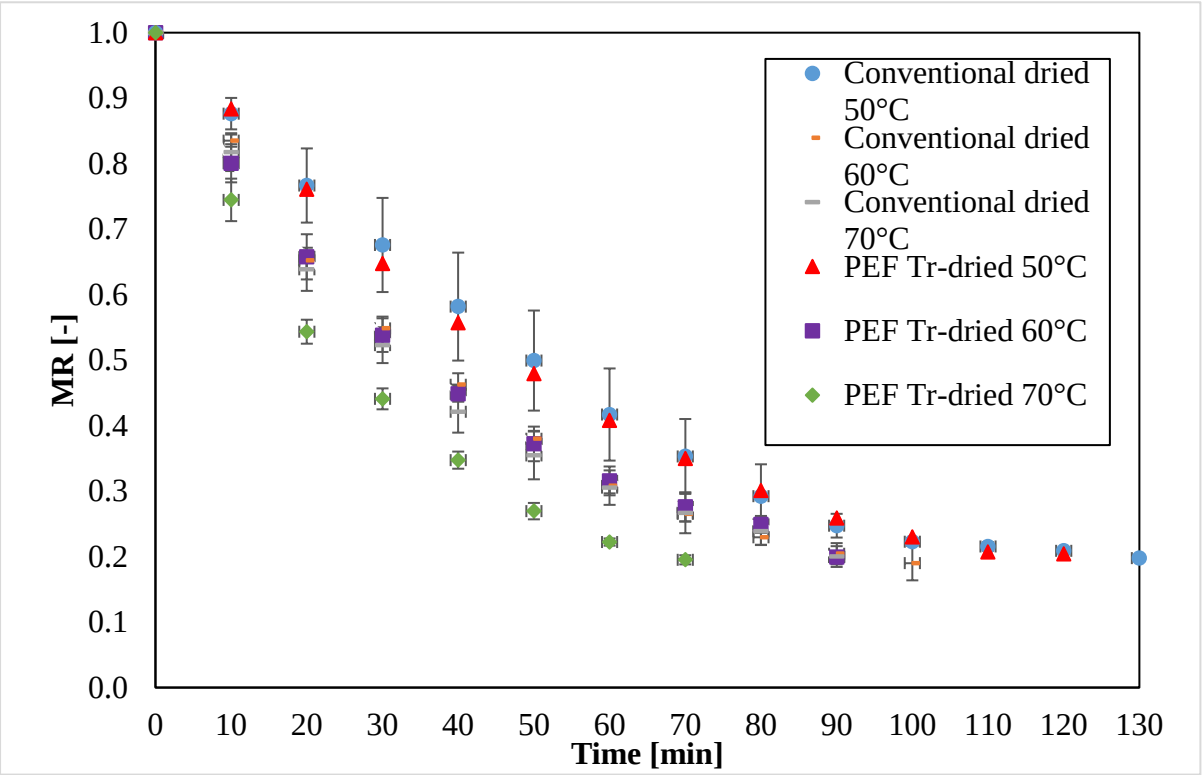


Figure 18. Drying kinetics of conventional and PEF treated parsnips.

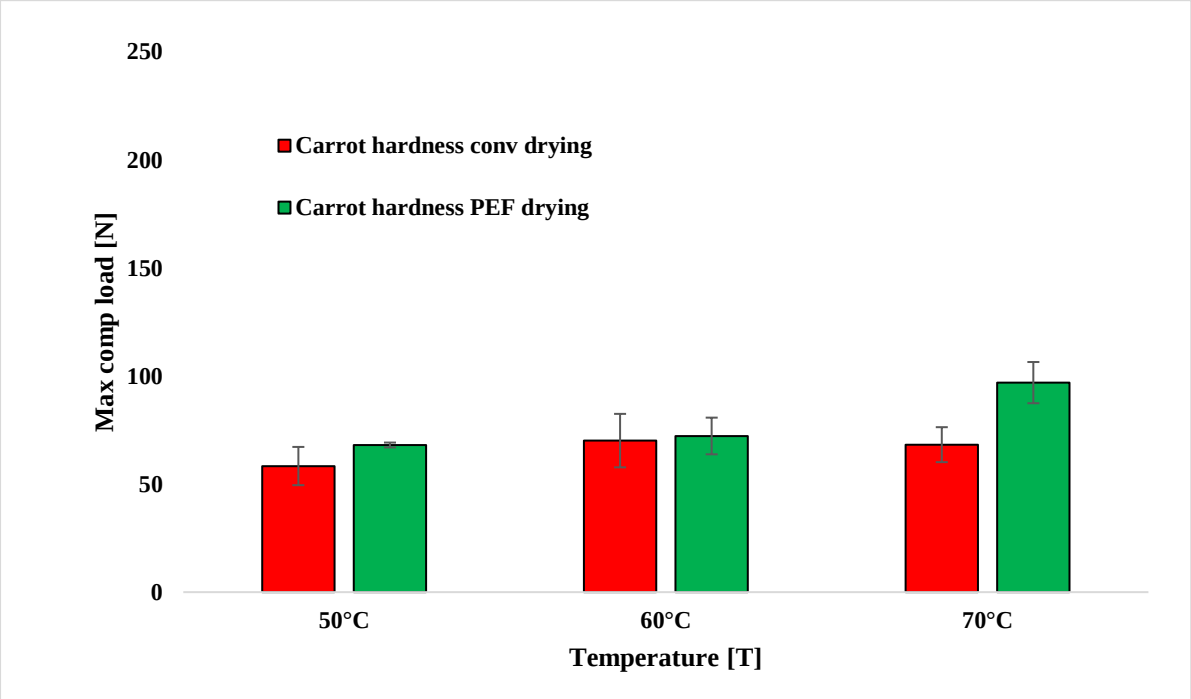


Figure 19. Texture test in conventional and PEF treated dried carrot samples.

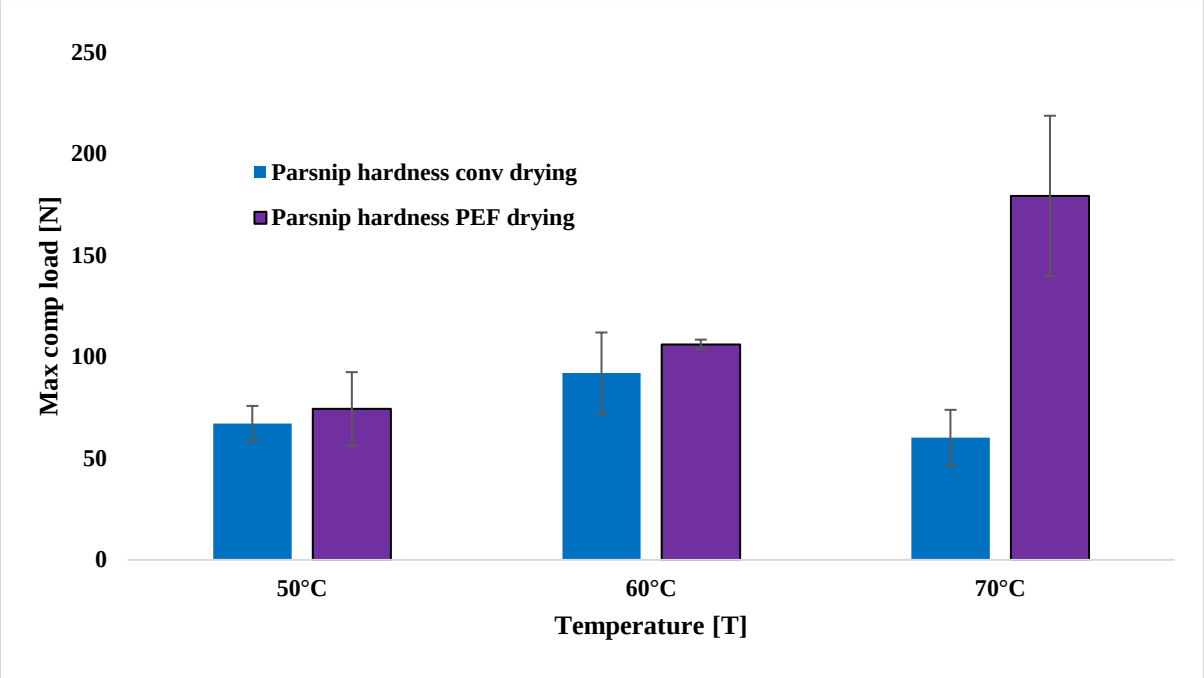


Figure 20. Texture test in conventional and PEF treated dried parsnip samples.

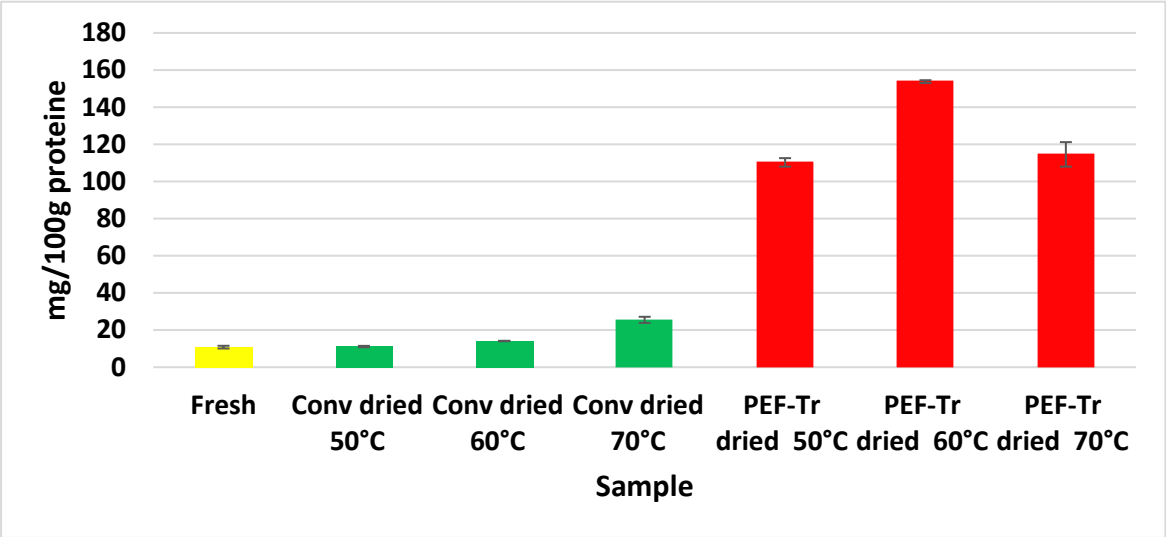


Figure 21. Furosine content in fresh, conventional and PEF treated dried parsnip samples.

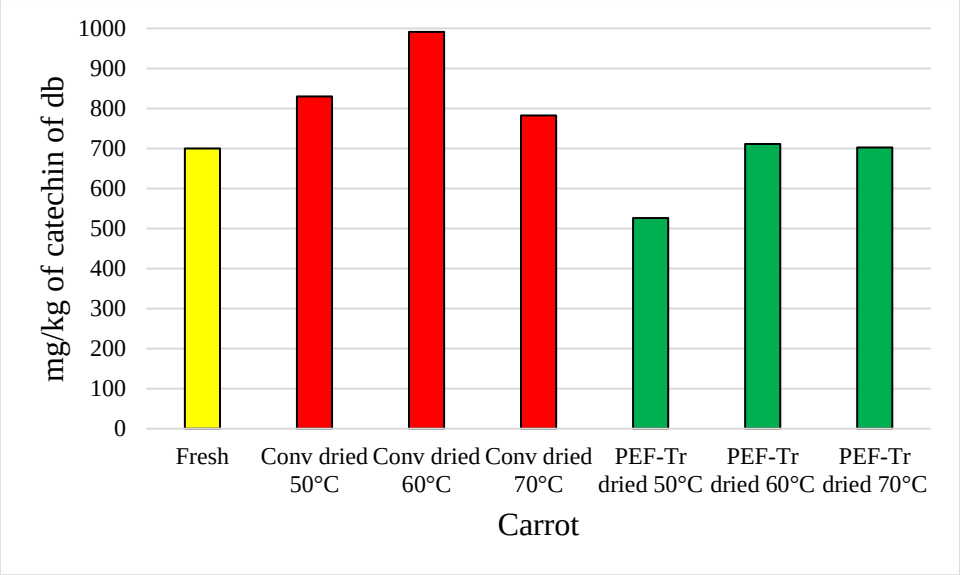


Figure 22. Total soluble phenolics (TSP) content in fresh, conventional and PEF treated dried carrot samples.

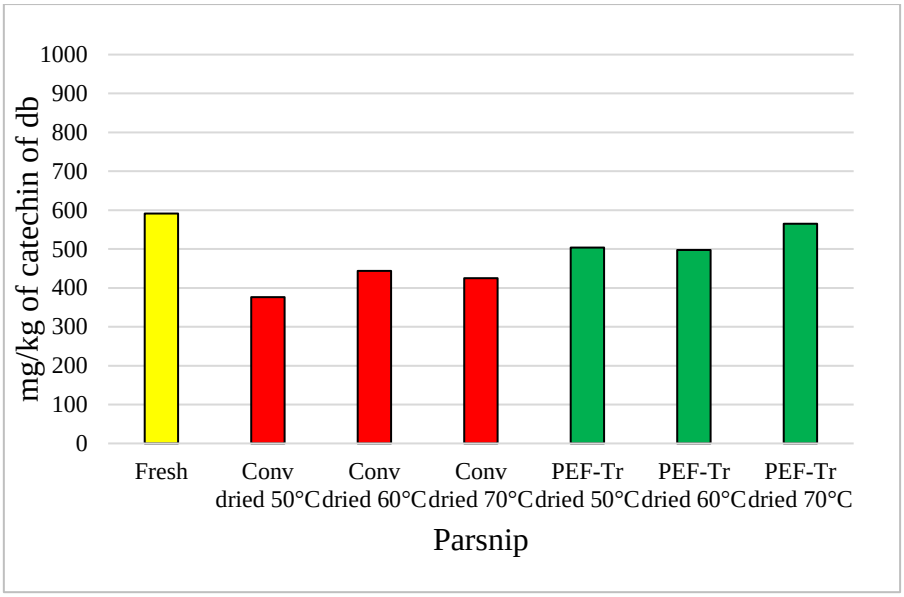


Figure 23. Total soluble phenolics (TSP) content in fresh, conventional and PEF treated dried parsnip samples.

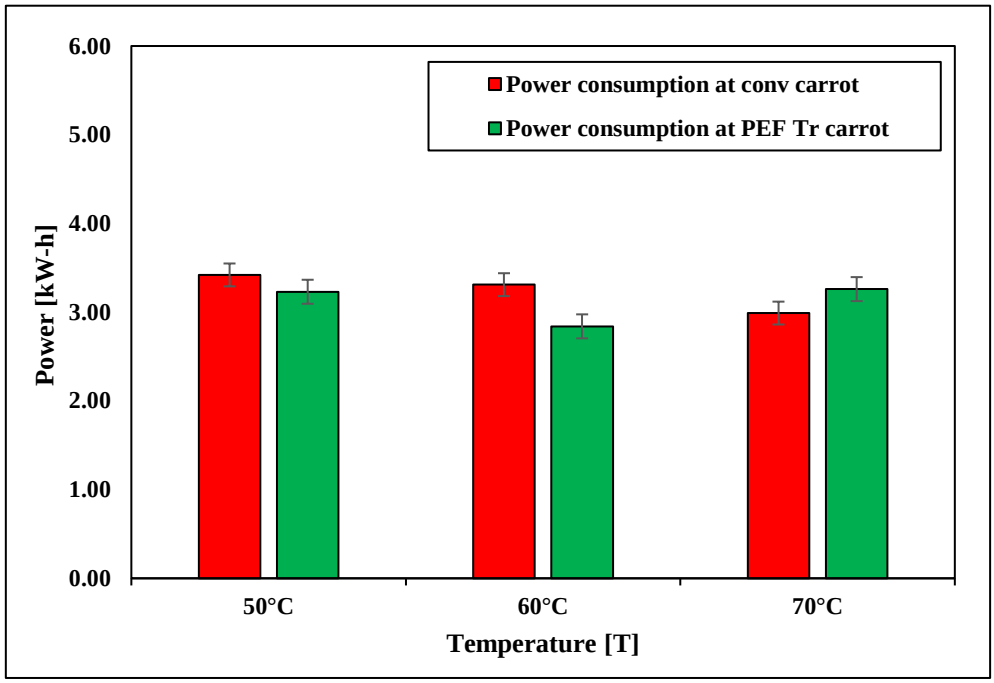


Figure 24. Energy consumption of conventional and PEF treated carrot samples during drying experiments.

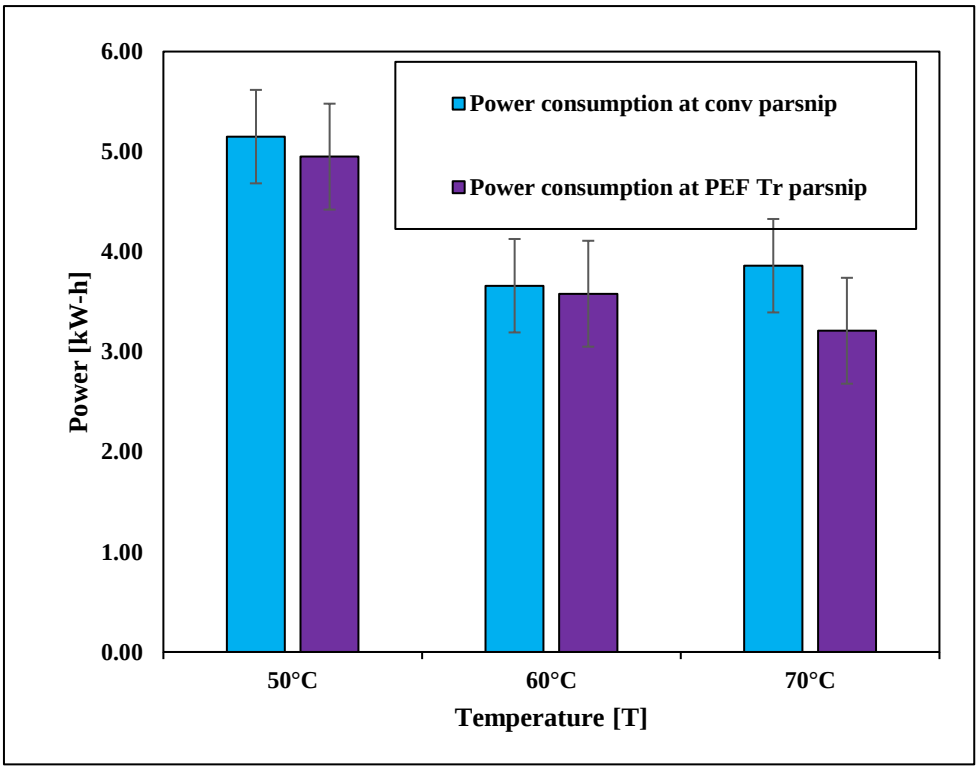


Figure 25. Energy consumption of conventional and PEF treated parsnip samples during drying experiments.

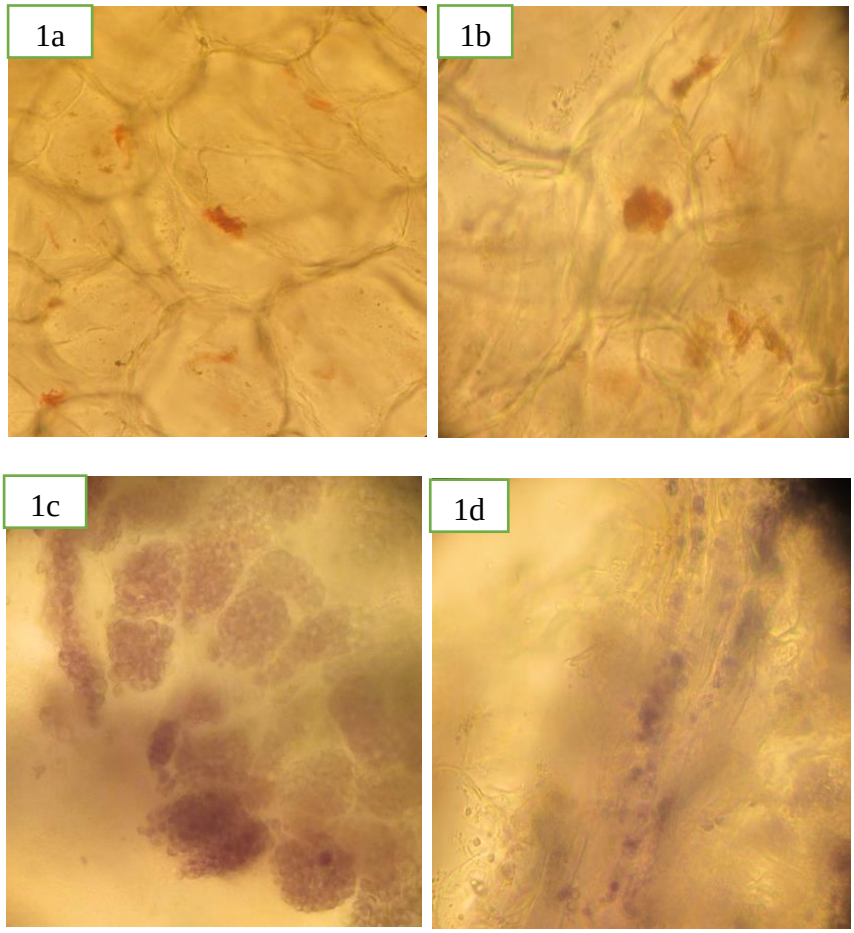


Figure 26. Microscopic images of carrot - fresh (a) and PEF pre-treated dried (b) – and of parsnip - fresh (c) and PEF pre-treated dried (d). The carotenoids and the starch granules are highlighted.

Table 1. Characteristic low and high frequency values for different biological material.

Biological material	Low frequency (kHz)	High frequency (MHz)
Large cells		
Animal muscle tissue	≤3	≥15
Fish tissue (mackerel or salmon)	≤3	≥3
Plant cells (apple, potato, or paprika)	≤5	≥5
Small cells		
Yeast cells (*S. cerevisiae*	≤50	≥25

Table 2. Electric field strength (EFS) and peak current during carrot treatment.

No. of Pulses	EFS average (kV/cm)	STD (kV/cm)	Peak Current (A)	STD (A)	Total Treatment Time (ms)
2	0.88	0.01	16.58	0.7	0.04
2	0.85	0.02	17.02	0.6	0.08
2	0.82	0.01	18.12	0.5	0.12
2	0.77	0.01	19.16	0.7	0.16
2	0.72	0.02	20.28	0.6	0.2
5	0.86	0.02	25.40	0.5	0.3
5	0.83	0.02	26.32	0.8	0.4
10	0.95	0.03	27.90	1.1	0.6
20	0.94	0.01	29.78	0.6	1.0
25	0.93	0.03	30.16	0.6	1.5
25	0.92	0.03	30.28	0.6	2.0
100	0.94	0.03	31.06	0.6	4.0
150	0.93	0.02	31.48	0.6	7.0
150	0.92	0.03	31.84	0.7	10.0
500	0.87	0.03	33.02	0.7	20.0

Table 3. Electric field strength (EFS) and peak current during parsnip treatment.

No. of Pulses	EFS average (kV/cm)	STD (kV/cm)	Current (A)	STD (A)	Total Treatment Time (ms)
2	0.89	0.05	17.78	0.62	0.04
2	0.84	0.04	18.78	0.78	0.08
2	0.83	0.03	19.00	0.70	0.12
2	0.81	0.04	19.16	0.60	0.16
2	0.80	0.04	19.42	0.71	0.20
15	0.97	0.07	24.54	1.49	0.50
15	0.95	0.06	24.86	1.52	0.80
15	1.04	0.04	25.62	1.49	1.10
15	1.03	0.04	27.60	1.29	1.40
15	1.01	0.04	28.60	0.75	1.70
15	0.97	0.05	29.18	0.91	2.00
100	0.99	0.04	30.00	0.94	4.00
200	0.96	0.04	30.66	0.98	8.00
100	0.95	0.03	30.98	0.61	10.00
500	0.70	0.35	32.73	1.10	20.00
1000	0.93	0.02	31.46	0.57	40.00
1000	0.84	0.03	33.68	0.81	60.00
2000	0.73	0.04	36.28	1.15	100.00
2000	0.67	0.04	37.92	0.93	140.00
3000	0.59	0.02	40.02	0.71	200.00

Table 4. Proximate composition of fresh and dried goji berries (g/100 g).

	Moisture[§]	**Fats**	**Proteins**	**Carbohydrates***	**Fibre**			**Ash**
					Soluble	**Insoluble**	**Total**	
Fresh	77.4±0.4	1.1±0.02	2.5±0.12	15.3	0.67±0.17	2.2±0.02	2.9	0.84±0.11
Dried	9.3±0.02	4.4±0.45	10.2±0.22	61.3	2.6±0.06	8.8±0.01	11.4	3.4±0.16

[§] mean ± standard deviation
*calculated by difference

Table 5. Average values of mineral elements in fresh and dried goji berries (mg/100 g).

	Fresh	**Dried**
Ca*	26.6 ± 4.90	101.3 ± 22.60
K	276.2 ± 41.00	881.9 ± 239.70
Mg	12.71 ± 2.80	45.9 ± 9.20
Na	57.3 ± 8.70	209.8 ± 72.30
P	48.4 ± 92.60	174.3 ± 32.10
Co	0.001	0.001
Cu	0.25 ± 0.038	0.83 ± 0.249
Fe	0.90 ± 0.219	3.40 ± 1.569
Mn	0.16 ± 0.032	0.51 ± 0.175
Zn	0.51 ± 0.117	1.53 ± 0.623
Se (µg/100g)	0.028 ± 0.008	0.067 ± 0.028
Mo (µg/100g)	0.00	0.00

* mean ± standard deviation

Table 6. Percentage contribution to the RDA of minerals in fresh and dried goji berries.

	Reg. RDA mg/day	Fresh % RDA	Dried % RDA	Dried % RDA x 30g
Ca	800	3	13	4
K	2000	14	44	13
Mg	375	3	12	4
P	700	7	25	7
Cu	1	25	84	25
Fe	14	6	24	7
Mn	2	8	26	8
Zn	10	5	15	5
Se (µg)	55	0	0	0

Table 7. Average carotenoid amounts in fresh and dried goji berries (mg/100 g).

	Fresh		Dried	
	Unsaponified	**Saponified**	**Unsaponified**	**Saponified**
Lutein*	0.0	1.1 ± 0.02	0.0	5.7 ± 0.44
Zeaxanthin	0.0	53.8 ± 0.82	0.0	186.0 ± 3.80
β–cryptoxanthin	0.0	2.3 ± 0.25	0.0	6.1 ± 0.14
Zeaxanthindipalmitate	47.8 ± 2.32	0.0	158.8 ± 1.53	0.0
Esters	8.5 ± 1.24	0.0	24.5 ± 2.30	0.0
β–carotene	0.2 ± 0.01	0.1 ± 0.01	0.9 ± 0.90	1.0 ± 0.20
Total carotenoids	56.4 ± 1.23	57.3 ± 0.80	184.2 ± 1.52	198.8 ± 3.80

***** mean ± standard deviation (mg/100g).

Table 8. Average tocopherol amounts in fresh and dried goji berries (mg/100 g).

	Fresh	Dried
α–tocopherol*	1.4 ± 0.10	5.5 ± 0.48
β -tocopherol	1.0 ± 0.01	4.2 ± 0.04
Total tocopherols	2.4 ± 0.04	9.7 ± 0.20
Tocopherol Equivalent (TE) [§]	2	8

* mean ± standard deviation (mg/100g).
[§] calculated as in Sheppard et al., 1993.

Table 9. Color parameters for both pre-treated and untreated fresh and dried samples.

Sample	L*	a*	b*	Hue angle	Chroma
Fresh	51.38 ±3.48[a]	30.95 ±2.25[a]	42.42±4.46[a]	53.82±1.98[a]	52.53±4.66[a]
Fresh after pretreatment	51.93 ±0.39[a]	31.28 ±2.76[a]	38.90.±5.66[a]	51.87±1.65[a]	49.53±4.13[a]
UTR dried	39.88 ±2.29[c]	12.47 ±2.31[c]	5.77 ±1.31[c]	24.73±2.07[c]	13.74±2.61[c]
Tr-Abr dried	44.17±1.85[b]	27.81±1.32[c]	23.80 ±1.33[b]	40.58±1.55[b]	36.61±1.59[b]

Table 10. Proximate composition of fresh and dried carrot and parsnip (g/100 g).

Carrot								
	Moisture[§]	**Fats**	**Proteins**	**Carbohydrates***	**Starch**		**Fibre Total**	**Ash**
					Resistant	**Non resistant**		
Fresh	85.3±0.15	0.3±0.08	0.7±0.03	7.6	0.02±0.00	1.52±0.12	3.7±0.10	0.87±0.11
Dried	9.6±1.4	1.9±0.10	4.3±0.20	46.6	0.12±0.01	9.3 ± 0.25	22.9±0.15	5.3 ± 0.16
Parsnip								
Fresh	81.5±0.20	0.4±0.15	2.3±0.75	9.6	0.02±0.00	0.98±0.08	4.3±0.15	0.86±0.01
Dried	6.0±0.54	2.0±0.30	11.8±1.6	49.0	0.10±0.01	5.0 ±0.14	21.7±0.25	4.4 ±0.15

[§] mean ± standard deviation
*calculated by difference

Table 11. Comparison of drying time between conventional and PEF treated carrots and parsnips.

	Process time [min] to reach MR=20%					
Process	Conventional drying			PEF treated drying		
Drying temperature [°C]	50	60	70	50	60	70
Carrot	102±2.6	80±0.9	59±0.4	94±0.4	63±0.8	69±1.6
Parsnip	128±3.0	93±1.8	90±1.6	120±1.3	90±0.1	68±0.7

Table 12. Color changes after PEF treatment in carrot.

Sample	L	a	b	h (°)	Chroma, C*
Fresh after Pre-treatment	50.0±1.48[a]	21.03±5.92[a]	22.58±0.77[a]	47.79±8.17[a]	31.06±4.14[a]
PEF Tr-dried 50°C	56.6±0.37[b]	29.10±2.69[a]	20.91±1.85[a]	35.8±0.47[a]	35.8±3.25[a]
PEF Tr-dried 60°C	57.1±0.66[b]	30.51±3.37[a]	24.68±3.25[a]	39±2.77[a]	39.3±4.26[a]
PEF Tr-dried 70°C	57.1±0.95[b]	27.05±4.81[a]	22.77±4.68[a]	40±1.32[a]	35.4±6.66[a]
Conventional dried 50°C	63.5±1.56[c]	28.06±1.74[a]	24.98±0.75[a]	41.7±1.77[a]	37.6±1.51[a]
Conventional dried 60°C	61.3±0.58[c]	28.66±1.26[a]	24.45±0.99[a]	40.5±2.38[a]	37.7±0.34[a]
Conventional dried 70°C	62.0±1.09[c]	27.03±2.08[a]	24.63±1.54[a]	42.4±2.01[a]	36.6±2.25[a]

Table 13. Color changes after PEF treatment in parsnip.

Sample	L	a	b	h (°)	Chroma, C*
Fresh after Pre-treatment	65.86±0.60 [a]	-2.49±0.02[a]	16.33±0.44[a]	98.71±0.24[a]	16.52±0.43[a]
PEF Tr-dried 50°C	75.14±2.83 [a]	4.18±0.35[b]	17.14±2.49[a]	76.21±1.38[a]	17.64±2.48[a]
PEF Tr-dried 60°C	73.64±9.60 [a]	7.81±2.74[b]	10.67±9.42[a]	43.09±25.9[b]	13.72±8.75[a]
PEF Tr-dried 70°C	74.52±0.64 [a]	3.74±1.00[b]	14.54±5.72[a]	73.02±11.4[a]	15.16±5.19[a]
Conventional dried 50°C	78.77±4.69 [a]	-1.34±0.33[a]	13.48±1.22[a]	95.81±1.65[a]	13.55±1.2[a]
Conventional dried 60°C	80.51±3.16 [a]	-1.04±0.28[a]	16.32±1.59[a]	93.75±1.21[a]	16.36±1.58[a]
Conventional dried 70°C	81.35±1.82 [a]	-0.58±0.79[a]	13.52±2.57[a]	92.39±3.42[a]	13.54±2.57[a]

Table 14. Average carotenoid amounts in fresh lyophilized carrot.

	Fresh lyophilized carrot
	average s.s. mg/Kg
13cis β-carotene	81.4±4.6[a]
α-carotene	127.1±3.3[a]
β-carotene	677.6±50.9[a]
9cis β-carotene	13.4±0.0[a]
Phytoene	96.1±1.7[a]
Total carotenoid	983.9±52.1[a]

Table 15. Average carotenoid amounts in conventional and dried carrot at 50°C.

	Carrot 50°C conventional	**Carrot 50°C PEF**
	average s.s. mg/Kg	average s.s. mg/Kg
13cis β-carotene	41.4±2.6[b]	38.7±4.2[b]
α-carotene	139.3 ±4.8[a]	108.3±5.5[b]
β-carotene	684.0±47.0[a]	464.1±14.2[b]
9cis β-carotene	11.7±0.9[b]	6.4±0.6[c]
Phytoene	141.0±14.6[b]	91.8±6.4[a]
Total carotenoid	1008.9±67.1[a]	714.7±23.9[b]

Table 16. Average carotenoid amounts in conventional and dried carrot at 60°C.

	Carrot 60°C conventional	**Carrot 60°C PEF**
	average s.s. mg/Kg	average s.s. mg/Kg
13cis β-carotene	50.0±1.9[c]	47.5±3.8[c]
α-carotene	139.0±13.4[a]	118.0±11.5[a]
β-carotene	653.8±59.4[a]	562.2±56.5[a]
9cis β-carotene	4.4±0.4[c]	5.8±0.1[c]
Phytoene	138.5±1.6[b]	124.6±12.6[d]
Total carotenoid	988.4±136.2[a]	828.2±78.0[b]

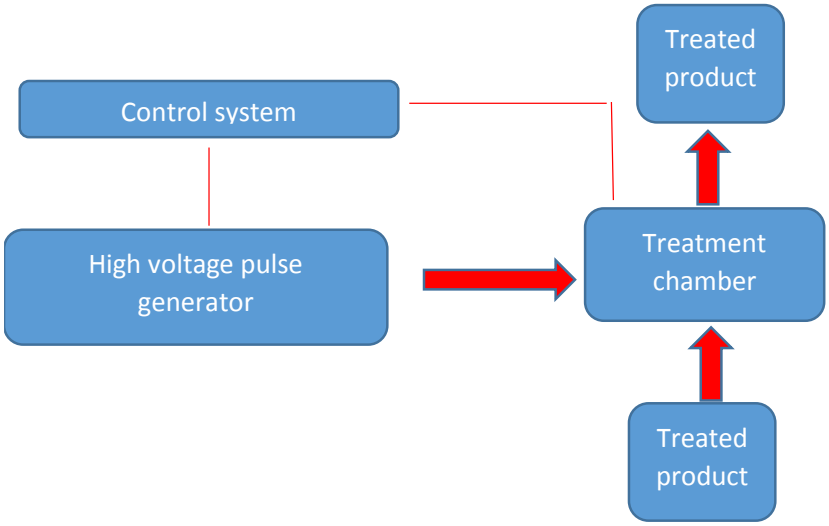
Table 17. Average carotenoid amounts in conventional and dried carrot at 70°C.

	Carrot 70°C conventional	Carrot 70°C PEF
	average s.s. mg/Kg	average s.s. mg/Kg
13cis β-carotene	73.7±1.3[c]	49.5±5.6[c]
α-carotene	139.2±5.0[a]	93.4±5.4[c]
β-carotene	690.2±63.1[a]	486.0±57.9[b]
9cis β-carotene	6.9±0.6[c]	5.0±0.4[c]
Phytoene	144.4±13.6[b]	87.9±6.6[a]
Total carotenoid	1054.9±78.7[a]	719.5±75.3[b]

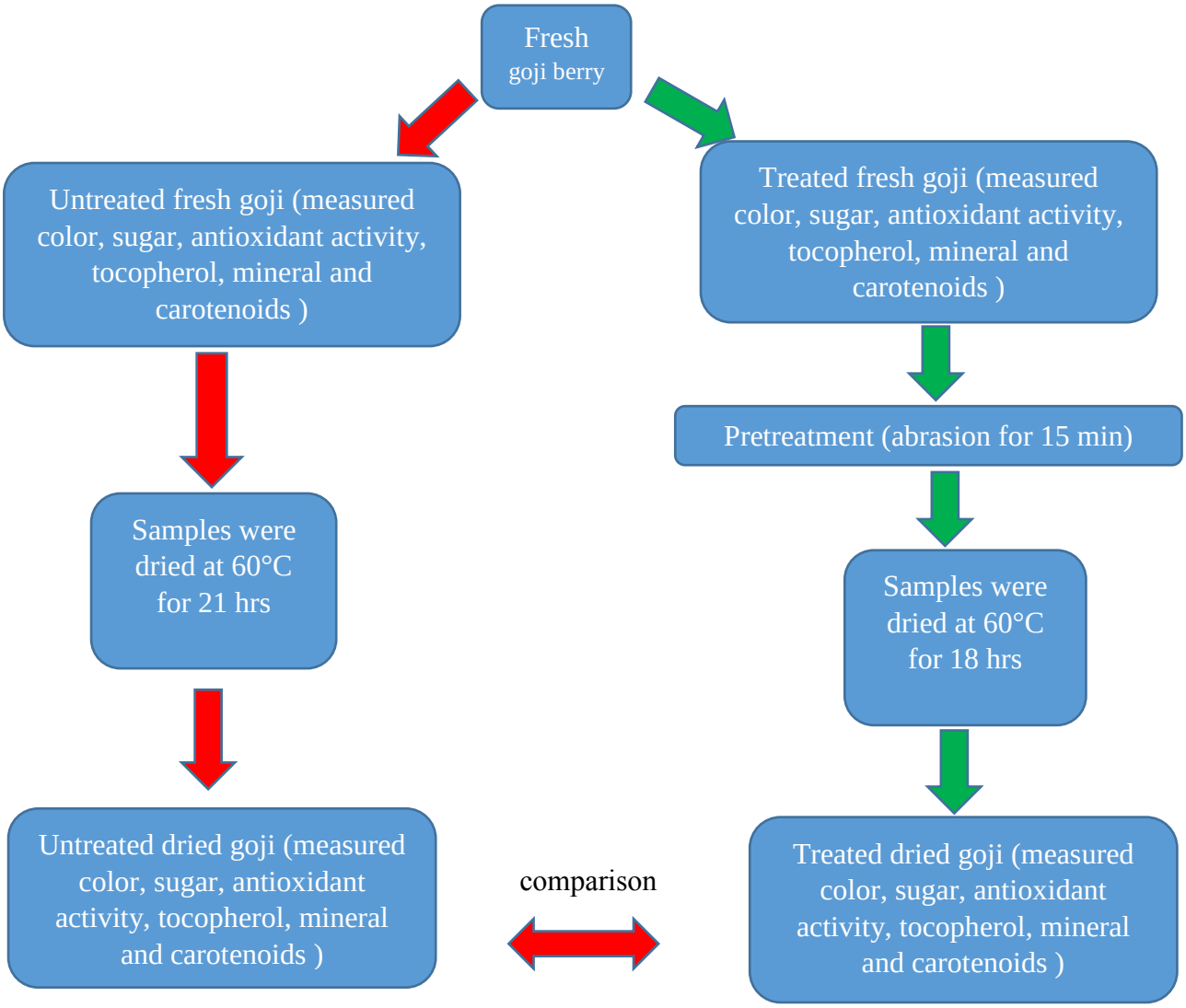
Table 18. Comparison of effective diffusivity between conventional and PEF treated carrots and parsnips.

Effective Diffusivity of carrot, Deff [m^2/s]			
		Carrot	
Drying temperature	50°C	60°C	70°C
Conventional drying	$3.4 \cdot 10^{-11}$-$9.9 \cdot 10^{-11}$	$1.1 \cdot 10^{-10}$-$9.7 \cdot 10^{-11}$	$1.1 \cdot 10^{-10}$-$6.0 \cdot 10^{-12}$
PEF treated drying	$1.0 \cdot 10^{-10}$-$9.9 \cdot 10^{-11}$	$1.1 \cdot 10^{-10}$-$7.6 \cdot 10^{-11}$	$1.1 \cdot 10^{-10}$-$8.4 \cdot 10^{-11}$
		Parsnip	
Drying temperature	50°C	60°C	70°C
Conventional drying	$1.9 \cdot 10^{-11}$-$8.9 \cdot 10^{-11}$	$1.0 \cdot 10^{-10}$-$9.8 \cdot 10^{-11}$	$1.0 \cdot 10^{-10}$-$9.9 \cdot 10^{-11}$
PEF treated drying	$2.1 \cdot 10^{-11}$-$8.6 \cdot 10^{-11}$	$1.0 \cdot 10^{-10}$-$9.5 \cdot 10^{-11}$	$1.1 \cdot 10^{-10}$-$5.7 \cdot 10^{-11}$

1. Flow diagram: PEF processing system.



2. Flow diagram: Methodology for goji berry.



3. Flow diagram: Methodology of carrot and parsnip.