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The Future of Pulsed Electric Fields in Microgreen Production: A Comparative Study

Mafalda de Aguiar Macedo e Ferreira dos Santos

Dissertação – Versão Final

Mestrado em Engenharia Agronómica

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CHAVE MÓVEL

“To each, there comes in their lifetime a special moment when he is figuratively tapped on the
shoulder and offered a chance
to do a very special thing,
unique to him and fitted to his talents.

What a tragedy if that moment finds him unprepared or unqualified
for that which could have been his finest hour.”
Winston S. Churchill

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ABSTRACT

Global demographic trends and increasing societal awareness of the need for sustainable, functional, and safe food products pose significant challenges to achieving the *Zero Hunger* goal proposed in the United Nations' Agenda 2030. This reinforces the urgency of addressing contemporary issues in food systems. A combination of heritage farming practices and innovative technologies is crucial to overcoming these challenges. Pulsed Electric Fields (PEF), a sustainable, non-thermal, and purely physical technology, has shown potential to optimise and modulate germination, growth, and the physicochemical and nutritional properties of plants derived from *PEFprimed* seeds

This study investigates the effects of PEF seed priming on the growth and quality of microgreens of three species: Beetroot, Arugula, and Basil. A comparative analysis was conducted using Control and three PEF protocols (PEFA: 2 kV/cm, PEFB: 3 kV/cm, and PEFC: 4 kV/cm), examining physiological, biochemical, and sensory parameters. Key data collected during the first five days of germination included water uptake, electrical conductivity, radicle length, and germination indices. Following harvest, microgreens were analysed for Total Phenolic Compounds (TPC), antioxidant capacity (DPPH & ABTS), Total Soluble Solids (TSS), pigments (Chlorophylls & Carotenoids), and nutritional composition (macronutrients and amino acids) via FT-NIR.

Photosynthetic pigments increased in Basil microgreens but decreased in Beetroot and Arugula. Sensory analysis was also conducted, using Quantitative Descriptive Analysis (QDA). Beetroot showed up to a +15% increase in TPC for PEFB and up to +12% improvement in antioxidant capacity (DPPH; PEFC), especially with ultrasound extraction (US). Arugula presented a +5% increase in DPPH antioxidant activity (PEFC). TSS increased by up to +7.5% in Beetroot (PEFC) but decreased by around 3 to 4% in Arugula. Extraction efficiency differed between Magnetic Stirring (MS) and US, with ultrasound generally yielding higher rates ($p<0.001$). In Basil, DPPH and ABTS scavenging assays presented distinct results, suggesting that the impact on ABTS-reactive compounds is not as dependent on PEF seed *electropriming* as DPPH.

With respect to Nutritional Content, *Fat* and *Protein* increased in Beetroot (Fat: +41%, Protein: +34%) and Arugula (Fat: +91%, Protein: +11%) treated with PEFC. PEFB led to an increase in *Starch* in all species. *Crude Fiber* and *Neutral Detergent Fiber* decreased amongst all species. *Methionine* rose by 100% in Beetroot treated with PEFC. Although not statistically significant, sensory analysis revealed small increases in *Sweet* (Beetroot) and *Aroma Intensity* (Arugula), along with a reduced perception of the descriptor *Hot* in Arugula.

Results demonstrated that PEF treatments significantly influenced water uptake, electrical conductivity, radicle length, and nutritional composition. Species-specific responses to different PEF protocols were observed. Optimal protocols appear to be PEFC for Beetroot, PEFB for Arugula, and PEFA/B for Basil. These findings not only suggest the potential of PEF as a tool for enhancing/modulating microgreen quality and nutritional profiles but also open the door for its utilisation in a wider agricultural context.

KEYWORDS: Pulsed Electric Fields, Microgreens, Seed Priming, Electrostimulation, Antioxidant Activity, Chlorophylls, FT-NIR, Sensory Analysis

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LIST OF ABBREVIATIONS

MS - Magnetic Stirring

$\boldsymbol{C_a}$ - Chlorophyll a

$\boldsymbol{C_b}$ - Chlorophyll b

$\boldsymbol{C_{a+b}}$ – Total Chlorophyll

$\boldsymbol{C_{x+c}}$ – Total Carotenoids

$\boldsymbol{CGI}$ – Cumulative Germination Index

$\boldsymbol{CV}$ – Kotowski's Coefficient of Velocity Index

$\boldsymbol{DGI}$ – Daily Germination Index

$\boldsymbol{E}$ – Electrical Field Strength

ESAV – Agrarian School of Viseu

FT-NIR – Fourier Transform Near-Infrared Reflectance

PEF – Pulsed Electric Fields

$\boldsymbol{SVI}$ - Seed Vigour Index

TPC - Total Phenolic Compounds

TSS - Total Soluble Solids

US - Ultrasound

$\boldsymbol{W_s}$ – Specific Energy

I. INTRODUCTION – STATE OF THE ART

1. SOCIAL & SCIENTIFIC FRAMEWORK

Nowadays, the pursuit of sustainable, healthier, and functional foods, along with great organoleptic properties, is highly prevalent in our societies (Demirtas, 2019; Rezai *et al.*, 2012). The increased consumer consciousness regarding this matter, coupled with Humanity's unmet food requirements, is catapulting the interest of producers and the scientific community towards the emergence, development, and application of new processing technologies in the Agrifood sector. These new technologies, such as Pulsed Electric Fields (PEF), need to demonstrate the potential to enhance the efficiency & sustainability of pre-established methods and techniques, promote the reduction or substitution of production factors, optimise the production of food assets, or even improve nutraceutical properties, while preserving organoleptic characteristics (van Boekel *et al.*, 2010). In addition, there's an urgency in finding solutions given that agricultural & food sustainability will be harder to reach due to already present and future challenges associated with demographic changes, industrialisation of agriculture, land-use conflicts, environmental issues, food supply & security disparity between 1st world and 3rd world countries, and increase of health costs associated with diet and lifestyle health problems in young age groups (Reisch *et al.*, 2013; Premanandh, 2011;).

The United Nations predicts that the world's population will continue to grow, reaching 10.4 billion by 2100 (Dorling, 2021). FAO estimates that by 2030, 8% of the global population, equivalent to 670 million people, will suffer from undernourishment (FAO, 2022) (Figure 1).

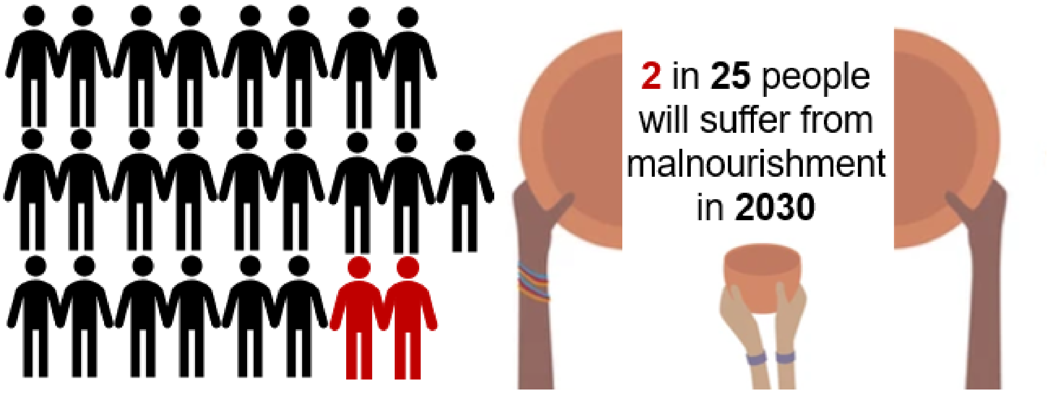


Figure 1. Representative estimates of malnourishment in 2030 (Photo of the Author, adapted from FAO, 2022).

Given the current circumstances, although progress has been made, achieving the Zero Hunger goal proposed by the United Nations' Agenda 2030 and its Sustainable Development Goals (SDGs) is becoming increasingly challenging (FAO, 2022) (Figure 2). There is a growing urgency among scientists and governments to tackle contemporary challenges regarding food insecurity, losses and waste, and escalating food requirements, while promoting sustainable agricultural practices. It is important to notice that the Agrifood industry not only serves as a vital provider of nourishment but also plays a pivotal role in contributing to the socio-economic stability of most countries (Miranda *et al.*, 2019, FAO, 2017) (Figure 3). Thus, a combination of heritage farming practices and innovative technologies and strategies is crucial to surpass these challenges.

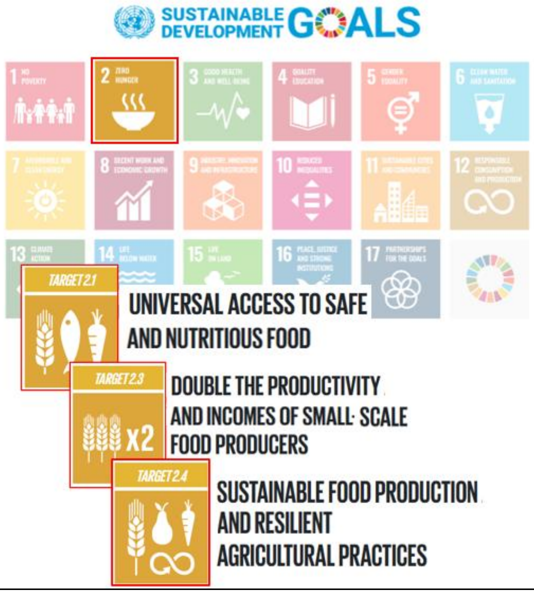
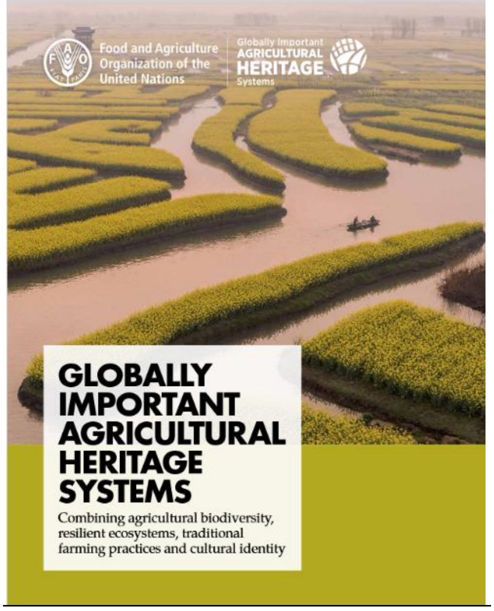


Figure 2. Agenda 2030's SDGs and End Hunger Targets (Adapted from: FAO, 2017).


Food and Agriculture Organization of the United Nations

Globally Important AGRICULTURAL HERITAGE Systems

GLOBALLY IMPORTANT AGRICULTURAL HERITAGE SYSTEMS

Combining agricultural biodiversity, resilient ecosystems, traditional farming practices and cultural identity

Figure 3. FAO Report on Globally GIAHS (In: FAO.org).

While the 1st mention of Microgreen utilisation is reported to the 1980s by chefs for culinary reasons, it has been low-profile (Treadwell *et al.*, 2010). Recently, the consumption of germinated seedlings, namely sprouts, baby greens, and microgreens, has been revitalised. In fact, according to *Research and Markets*, the microgreens market is expected to grow from $2.14 billion in 2024 to $2.4 billion in 2025, with a compound annual growth rate (CAGR) of 12.2%. Rapid growth is anticipated in the coming years, reaching $3.93 billion by 2029, with a CAGR of 13.1% (Research and Market, 2025). It is believed that such food products might have the potential to positively contribute to the resolution of the previously mentioned issues. It's also important to mention that while assessing the viability of new technologies and strategies capable of improving the germination rate and potentially enhancing the nutritional value of these foods, the short period between sowing and harvesting offers the advantage of obtaining faster conclusions that can be extrapolated and further studied in other agricultural contexts. In this work, we will focus on the microgreen production.

One of the technologies with the potential to optimise the production of vegetable goods, such as microgreens, and increase the presence of bioactive compounds in these food products is Pulsed Electric Fields (PEF).

Seed imbibition and germination can be modelled by the potential effects of PEF application on biochemical and physiological changes that occur during these stages. Furthermore, some research teams are also suggesting the possibility of applying PEF as a pre-sowing technique, not only with the objectives mentioned above, but also as a suppressant of pathogenic and spoilage microbiota, which can affect crops, shelf-life, and food safety (Evrendilek *et al.*, 2019).

2. MICROGREENS

2.1 MICROGREENS VS GERMINATED SEEDLINGS

Humans have been consuming germinating seedlings of various plant species since the dawn of time, due to their richness in proteins, vitamins, and minerals, which makes them a highly nutritious food source, when in comparison with their matured versions.

As mentioned earlier, germinated seedlings can be grouped into three different groups per their developmental stage:

a) Sprouts: totally or partially germinated seeds with underdeveloped cotyledons, lacking true leaves. They may or may not have chlorophyll present (Figure 4).
b) Microgreens: typically harvested without roots, with fully developed non-senescent cotyledons and/or one or two true leaves (Figure 5).
c) Baby greens: generally, baby greens have more than two true leaves at the time of harvest and are not collected for their fully developed cotyledons (Verlinden, 2020) (Figure 6).


Figure 4. Mung Bean Sprouts. (*Author:* Photobeast [野獣同輩], via Wikimedia Commons. Licence CC BY-SA 4.0., 2022)


Figure 5. Radish Microgreens (*In:* paperpot.co)


Figure 6. Baby Greens: Baby Spinach (*In:* theveganatlas.com)

However, these definitions might vary depending on the author, being only recently a legal definition has been attributed to “sprouts” and “baby leaf” terms, in part due to the relatively higher risk of microbial contamination associated with the consumption of sprouts, as will be further mentioned. Thus, the European Union (EU) was compelled to establish a legal definition for this germinated seedling type through Commission Implementing Regulation (EU) 208/2013 (European Union, 2013). The regulation defines it as follows: “the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves, and intended to be consumed whole, including the seed.” In 2014, “baby leaf” also gained a legal definition, being as follows: the young leaves and petioles of any crops (including *Brassica*) harvested up to 8 true leaves stage (European Union, 2014).

However, to the best of our knowledge, “Microgreens” remains without a standardised legal definition in the eyes of the European Union (EU). Di Gioia & Santamaria define microgreens as young and tender seedlings cultivated from a variety of vegetable, herbaceous, aromatic, and wild edible plant species (Di Gioia & Santamaria, 2015b).

While microgreens have been used for centuries, they are currently gaining renewed attention, considering their higher concentration of minerals, such as Ca, Mg, Mn & Zn, and lower nitrate content when in comparison to their mature versions (Kyriacou *et al.*, 2016; Pinto *et al.*, 2014; Xiao *et al.*, 2012). New discoveries highlighting their high levels of bioactive metabolites, such as β-carotene, and earned them the title of functional food (Leong *et al.*, 2016; Márton *et* *al.*, 2010). Not only that, but when in comparison with their counterparts, mainly to sprouts, microgreens pose as a better alternative from a Food Security and Quality point of view. For instance, sprouts growing conditions are usually associated with a dark, highly humid, and warm environment, perfect for microbial development, which unfortunately was already linked to breaches in Food Security and led to foodborne disease outbreaks In Food Quality terms, microgreens might pose as a more interesting alternative to consumers, due to vibrant colours and varied shapes and textures, as well as to their stronger flavour properties (Ebert, 2012). All these factors demonstrate the potential for microgreens to be classified as a superfood.

2.2 PRODUCTION: FROM SOWING TO HARVEST

2.2.1 SPECIES SELECTION

One of the points of interest associated with the cultivation of microgreens is the vast number of species able to be produced as Microgreens. From vegetables, to cereals, pseudocereals, legumes, herbs, and wild plants can be used to produce microgreens (Di Gioia *et al.*, 2017; Kyriacou *et al.*, 2016). The only exception associates with species that present toxicity at seed & seedling stage, as it is the case of *Solanaceae* family, which includes genus as *Solanum* (potato, tomato, eggplant), *Capsicum* (chili pepper) e *Nicotiana*, due to the presence of alkaloids, such as glycoalkaloids, nicotine and capsaicinoids (Pomilio *et al.*, 2008).

The most common microgreens are usually associated with the *Brassicaceae* family, including species from the genera *Brassica*, *Raphanus*, and *Eruca*, such as broccoli, mustard, radish, arugula, cabbage, and kale (Alloggia *et al.*, 2023). In addition, other traditional wild and vegetable species from families such as *Amaranthaceae* (beets and swiss chard), *Apiaceae* (carrot, onion and leek), *Asteraceae* (lettuce and chicory), *Poaceae* (barley, wheat), *Oleaceae* (sunflower) and *Lamiaceae* (basil, cilantro, dill) are commonly used (Figures 7 & 8) (Di Gioia *et al.*, 2023; Benincasa *et al.*, 2019; Di Gioia & Santamaria, 2015a).

Figure 7. Beetroot Microgreens (Photo of the Author),

Figure 8. Arugula Microgreens with a detail: an albino plantlet (Photo of the Author)

Due to this fact, microgreens can not only pose a vast array of attractive colours, flavours, and shapes, which sometimes grants them the epithet “vegetable confetti”, but also be conjugated and actively contribute to a complete dietary regimen due to their rich density of phytonutrients and bioactive compounds (Bhaswant *et al.*, 2023).

2.2.2 GROWING MEDIUM & TRAYS

Microgreens production is highly adaptable, as they can be grown in both soil and alternative media, in small trays under different environmental conditions, making them easy to cultivate in limited spaces. This makes them an ideal option for home growers, urban producers, and large-scale production systems, including greenhouses and vertical farming.

In terms of growing media, microgreen cultivation can be divided into two main groups:

a) Soilless media, which can be of organic nature (such as coconut coir, peat moss, wood fibre - including sawdust or tree bark - and hemp or jute mats) or inorganic nature (such as rockwool, perlite, and vermiculite).
b) Soil-based media, which involves the use of traditional soil-based substrates, such as potting or germination soil and peat (Saleh et al., 2022; Di Gioia, et al., 2017a; Di Gioia & Santamaria, 2015a; Murphy et al., 2010).

Additionally, growers can opt for either the selection of solid growing medium constituted by a balanced mixture of different substrates (i.e., 75% white sphagnum peat +25% vermiculite) (Hoang & Vũ, 2022) or hydroponic production (Chen *et al.*, 2020). The selection of growing medium is particularly important in microgreen production. Di Gioia and Dubey demonstrated that the growing medium significantly affects not only yield, quality, and microbiota population, but also the economic and environmental sustainability of the production process (Dubey *et al.*, 2024; Di Gioia *et al.*, 2017a). Thus, there is a consensus among scholars that an optimal growing medium should have the following key characteristics:

1. a high moisture retaining capacity (55-75%)
2. adequate aeration (Total Pore Space 20-30%)
3. pH in the range of 5.5 to 6.5
4. electrical conductivity >0.5dS/m^{-1}
5. free from microbial pathogens and contaminants
6. locally available, reusable, and cost-effective, as it can represent one of the main production costs (Dubey *et al.*, 2024; Mir *et al.*, 2017; Kyriacou *et al.*, 2016).

Currently, peat-based mix substrates and synthetic mats are the most commonly used for microgreen production. However, these are usually non-reusable and expensive. Therefore, the possibility of using “green” alternative substrates has been a recent focus of study, including recycled textile fibre (Di Gioia *et al.*, 2017a), biopolymer-based hydrogels (Du *et al.*, 2022), vermicompost (Dubey *et al.*, 2024) and spent mushroom compost (Poudel *et al.*, 2023).

Usually, the commercial production of microgreens occurs in plastic or organic trays with a height of 3-5cm, with small perforations within the bottom, to allow for proper drainage of excess water, which tends to compromise the production of microgreens due to the development of root diseases (Figure 9). Other options include microgreen growth elevated benches and the “floating method”, where polystyrene plug trays float in a nutritive and aerated solution, being the irrigation performed due to the perforated bottom of the trays (Di Gioia & Santamaria, 2015a).

2.2.3 SEED DENSITY

Selecting the most balanced sowing rate according to the desired objective is essential not only to maximise fresh yield per unit area and profitability, but also to maintain product quality (Nolan, 2019). Seed density is crop-specific, depending on factors such as average seed weight, estimated germination rate, and desired crop density (Di Gioia & Santamaria, 2015b; Murphy *et al.*, 2010). This parameter is particularly important due to the possible negative and positive outcomes of the selection of a sowing rate. For instance, overcrowding can lead to excessively elongated thin shoots, due to sunlight competition, restricted air circulation, capable of increasing fungal disease susceptibility, and higher production costs (Ntsoane *et al.*, 2023; Kyriacou *et al.*, 2016). In contrast, Signore *et al.* (2024) conducted a comparative study on three *Brassicaceae* species using sowing densities of 3 to 5 seeds/cm² and demonstrated that higher sowing intensities can enhance crop coverage and yield. However, at earlier harvest stages (11 vs. 14 days), they observed a delay in crop development equivalent to three days, as rapini seedlings grown at the lowest sowing density were more developed and had larger true leaves. However, the opposite was observed when using kale seeds. Other variables, such as temperature or season, can influence the seed rate. For example, Lerner *et al.* (2024) concluded that the ideal seed rate varied with the season, obtaining superior *Arugula* microgreen yield when using 150 g/m^2 of seeds in winter, as opposed to 175 g/m^2 in spring. This was also corroborated by Nolan's (2019) observations. She also observed the same for mustard, and the opposite for basil, which achieves higher yields in the higher temperatures of spring and summer.

Di Gioia & Santamaria (2015b) are prominent researchers in microgreen studies and generally advocate that for large-seeded species, such as peas, chickpeas, and sunflowers, the optimal sowing density is around 1 seed/cm², while for small-seeded species (e.g., arugula, watercress, and mustard), it can go up to a maximum of 4 seeds/cm².

Sowing Density Calculators available online allow the determination of seed rate considering variables such as species, sowing area and, sometimes, % of seed germinability. Before defining the seed density used for this study, several papers and Sowing Density Calculators were consulted to try to find common ground. Considering our tray area of 333 cm^2 (20 x 16.6cm), we used these online tools to calculate possible sowing rates for each variety used. The results obtained from the Microgreen Seed Calculator (Penn State University, version 20.1), developed by Di Gioia (2020), from specialised microgreen seed producers (MicroSeeds Hub, s/d; MP Seeds, s/d), a microgreen farm management software company that bases these results on industrial experience (Microgreen Manager, s/d), and from a random calculator available online (JSCalc.io, s/d), are as follows (Table 1):

Table 1. Advised Seed Weight (in grams / 333 cm^2) by available online tools

	Beetroot	Basil	Arugula
Penn State University	5.70	1.03	1.94
MP Seeds.eu	25.60	3.84	4.35
MicroSeeds Hub	11.58	2.57	4.12
Microgreen Manager	23	3	8
JSCalc.io	7.75	6.198	7.748

These divergent conclusions result in a non-existent seed rate standardisation, as growers and seed suppliers recommend varying sowing rates due to the challenge of determining the optimal density. However, a “rule of thumb” that everyone agrees on is that seeds should be evenly distributed in the tray, avoiding overlap and piling.

Optimising sowing densities requires a balance between maximising product yield and maintaining its quality, without disregarding different species sizes and necessities and growing conditions, such as temperature, humidity, type of cultivation and substrate. In fact, Nolan (2019) concluded that there was no correlation between yield and seed density; light exposure and season seem to be more influential regarding microgreen yield. In opposition, Choe *et al.* (2018) corroborated the existence of a relation between seed density and fresh weight yield, despite an individual shoot's fresh weight decrease. If an optimal seed density threshold is surpassed, the quality of microgreens is heavily compromised (Priti *et al.*, 2022).

The discrepancies are notorious and highlight the difficulty of defining an optimal sowing density, as different sources apply distinct methodologies and assumptions, each based on their own evidence and experiences.

2.2.4 GROWING CONDITIONS

Environmental conditions play a fundamental role in the growth, sensory quality, nutrient content, phytosanitary conditions, and yield of microgreens. Key factors include relative humidity, irrigation, light, temperature and ventilation.

Irrigation not only determines the plant’s water availability but also has significant implications for plant health and food safety, depending on the water source. The irrigation method can influence disease development due to excessive moisture on leaves, as well as the risk of pathogen contamination. Overhead irrigation, for example, increases the risk of pathogen dissemination (i.e. *E. coli*) and phytopathogens compared to drip or subsurface irrigation, as it results in greater contact between water and the shoots and leaves. Heavy metals present in irrigation water can be absorbed by the plants and enter the food chain supply; thus, water sources should also be monitored in this regard (Hosen *et al.*, 2024). While water deficit can lead to growth retardation or even crop loss, excess watering can contribute to the development of soilborne diseases, such as root rot (i.e. *Pythium spp.*), which in worst cases can lead to damping off and destruction of large sections in the tray (McGehee *et al.*, 2019).

Light can vary under different conditions, such as its quantity, measured by photoperiod and intensity (photon flux density), and quality, which is inherent to the used light spectrum. While microgreens are mostly produced in greenhouses, their production is also carried out under artificial light. Artificial lighting can serve as the main source or as a supplement, especially recommended if natural light is lower than 10–12 mol $day^{-1}m^{-2}$ (Verlinden, 2020; Craver *et al.*, 2017; Gerovac *et al.*, 2016). Both light quality and quantity are, similar to other parameters, species-dependent. For example, Nolan (2019) found that basil grew significantly better in full sun, while radish performed better in shaded conditions. Additionally, Mlinarić *et al.* (2020) found significantly higher levels of chlorophyll, carotenoids, total soluble phenols, and ascorbic acid in chia microgreens when grown under constant light intensity (100 μmol photons m^{-2} s^{-1}) for 48 hours, when compared to samples grown in the dark. Some species also require an initial blackout period of 1-3 days to kickstart germination by simulating natural soil conditions (Green, 2021).

Regarding light quality, different wavelengths can influence plant photosynthesis and metabolism differently, as plants possess photoreceptors capable of detecting specific wavelengths, from visible light red, blue and green, to infrared and ultraviolet radiation. Red and blue light are considered primary sources affecting nutrient accumulation due to their strong absorption by chlorophylls, which absorb mostly in the red and blue regions (663-642 nm and

430-453 nm) and carotenoids (448-454 nm) (Pescarini *et al.*, 2023). This is corroborated by the results obtained by Ying *et al.* (2021), who observed an increase in total anthocyanin content in arugula, red cabbage and kale, and in total phenolic content in kale and mustard microgreens, when applying a combination of 30%blue/70%red light ratio compared to a 5%blue/95%red ratio. Kong *et al.* (2020) also suggested that blue light was able to trigger a shade-avoidance response, leading to maximum elongation growth. Artificial light can be provided by various sources, including high-pressure sodium (HPS) lights, incandescent lights, fluorescent lights, and light-emitting diodes (LEDs) (Figure 9). LEDs are the most used, given their low heat transfer, energy efficiency, and the ability to easily modulate wavelength selection (Artés-Hernández *et al.*, 2022).

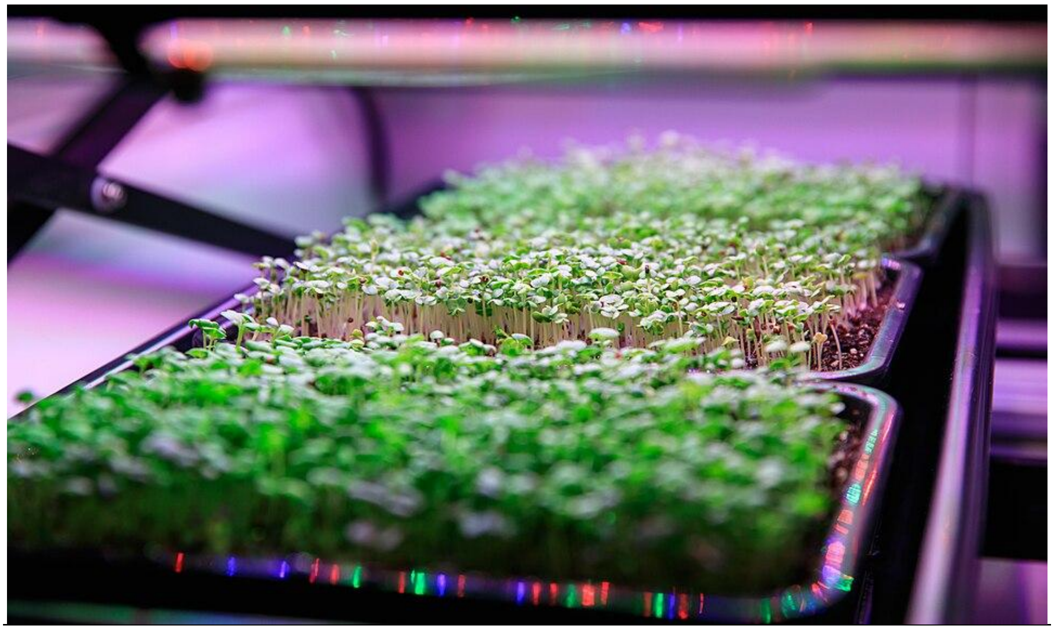


Figure 9. Microgreens being grown under artificial light at NASA's Kennedy Space Center's Space Station Processing Facility in Florida (©NASA / Watson, 2023).

Atmospheric characteristics, namely relative humidity (RH%), CO_2 concentration, and airflow, are important factors to consider. Maintaining an ideal relative humidity helps reduce water footprint by controlling crop transpiration, preventing plant desiccation, and influencing several physiological processes, such as photosynthesis, cell expansion, and plant growth, from early stages to maturity. For example, low relative humidity and high air velocity can lead to water stress and impact stomatal behaviour, while excess RH% and poor ventilation can contribute to the development of cryptogamic diseases, i.e., moulds in seeds and mildew (Chia & Lim, 2022; Talley *et al.*, 2002; Shibata *et al.*, 1995).

Similarly, to the other parameters, each species presents different requirements. However, it is generally considered that a favourable environment should present a RH range between 40 to 60%, and temperatures ranging from 15 to 32°C (Green, 2021; Toscano *et al.*, 2025). For instance, Verlinden (2020) refers to a linear rise in temperature from 14°C to 22°C to significantly reduce the harvest day by 35-40%. Additionally, proper air circulation is vital for disease prevention, as it helps regulate HR% and temperature by distributing them evenly across the crop surface (Bhaswant *et al.*, 2023).

2.2.5 HARVEST

When microgreens reach their optimal desired maturity, they can be harvested by cutting at the base of the hypocotyl, above the soil line, by hand-picking (with sterilised scissors), or by mechanical methods. Typically, they measure between 10 to 20 cm in length at this stage.

Considering their highly perishable and sensitive nature, microgreens are often sold in trays, allowing end users to collect them immediately before use (Verlinden, 2020; Di Gioia & Santamaria, 2015a; Treadwell *et al.*, 2010).

There is no clear consensus on the ideal harvest moment. Some authors (Di Gioia *et al.*, 2023; Mir *et al.*, 2017; Kyriacou *et al.*, 2016; Xiao *et al.*, 2015) suggest that microgreens should be harvested when cotyledons are fully expanded and turgid and the first pair of true leaves has emerged. Others (Pinto *et al.*, 2014; Sun *et al.*, 2013) argue that harvesting should occur before true leaves emerge. This process normally takes place 7 to 21 days after germination, varying greatly depending on crop species and desired maturation level (Kyriacou *et al.*, 2016).

Determining the optimal harvest time for microgreens should consider not only yield but also functional sensory quality (Ortiz *et al.*, 2024). Allowing plants to overdevelop may lead to undesired fibrousness, bitterness, and a reduction in nutritional richness. However, the ideal harvest period depends on the species and intended use, and there is a very limited bibliography addressing the ideal moment of harvest for different crops (Bhaswant *et al.*, 2023).

For hydroponically grown radish and broccoli microgreens, the ideal harvest stage was found to be when approximately 75% of plants had their 1st leaf emergence, based on yield, chlorophyll, antioxidant content, and post-harvest shelf-life (Ortiz *et al.*, 2024). Empirical knowledge from both microgreens' specialized websites and producers suggests that both radish and mustard microgreens are usually harvested at the cotyledon stage to maintain tenderness and mild spiciness. Similarly, sunflower and beet microgreens are typically harvested before true leaves emerge to avoid toughness and undesirable texture. Conversely, basil, coriander, and arugula are left to develop their 1st set of true leaves to enhance their characteristic flavour and aroma.

Recent studies provide further insights into harvest timing. Gök *et al.* (2024) compared microgreens harvested at the embryonic leaf stage vs the true leaf stage, concluding that dill and chia microgreens collected at the latter stages had higher total polyphenolic content and antioxidant capacity, whereas red beetroot and radish had higher nutritional content at the cotyledon stage. Similarly, Acharya *et al.* (2021) analysed chlorophylls, carotenoids, polyphenolic compounds, and ascorbic acid of beets microgreens from day 9 to day 21, concluding that the ideal harvest stage occurred at the 15th day of growth, from a nutritional perspective. Additionally, Kyriacou *et al.* (2021) found that mineral and phytochemical composition varied minimally between *Brassicacea* genotypes collected at the 1st and 2nd leaf stage. For low-yielding genotypes, a later harvest could be beneficial to maximize yield.

One thing is for sure: while the definition of microgreens and their optimal harvest remains unclear, microgreen harvesting shall take place after the sprouting phase and before the baby greens stage (Kyriacou *et al.*, 2021).

2.3 BIOACTIVE COMPOUNDS IN MICROGREENS

Before diving into the theme of this thesis, it is important to understand what characteristics define a bioactive compound. Bioactive compounds are often associated with secondary metabolites. Their difference regarding primary metabolites is that they do not directly participate in the plant's main physiological processes (photosynthesis, translocation, and respiration) and development. However, they are synthesized by plants, performing an important role in regulating those physiological processes (e.g. phytohormones) (Davies, 2010) and acting as a mechanism of defence against exogenous biotic and abiotic stresses, contributing to their competitiveness in their own environment (Twaij & Hasan, 2022; Guerriero *et al.*, 2018; Teoh, 2015; Patil *et al.*, 2009). Bioactive secondary compounds, also known as phytochemicals, are grouped according to their biosynthetic pathway, thus being defined into four different classes: phenolic compounds, terpenoids, nitrogen-based (e.g., alkaloids), and sulphur-based compounds (Saxena *et al.*, 2013). Phytochemicals present a vast array of characteristics that allow plants to better adapt to the surrounding environment. Phytoalexins endow plants with the ability to protect themselves from pathogens, having antibiotic, antifungal, and antiviral properties (e.g., resveratrol in *Vitis vinifera*) (Fernández-Mar *et al.*, 2012). Other plants can prevent animals and insects from consuming them by producing compounds with anti-herbivory properties or even by interfering with the reproductive cycle of animals due to their hormone-mimicking compounds (e.g., isoflavones produced by the *Fabaceae* family mimic oestrogen) (Bernhoft, 2010). For instance, insect herbivory causes mechanical damage that exacerbates the production of two of the most encountered alkaloids in human life today: *Coffea arabica* L.'s caffeine (1,3,7-N-trimethylxanthine) and *Nicotiana sp.* L.'s nicotine (1-methyl-2(3-pyridyl) pyrrolidone) (Harborne, 2007).

Allelopathic chemicals can interfere with plants' neighbouring peers, able to influence them positively or negatively, by posing as toxins that can inhibiting or induce seed germination and plant growth; *Zea Mays* is a great example, given it produces allelochemicals capable of inhibiting growth and even its pollen has the possibility of interfering in other species fruiting (Mushtaq *et al.*, 2020). UV protective compounds are produced as a defence mechanism against excessive UV radiation emitted by the sun. Phenolic compounds, anthocyanins, and carotenoids are some of the examples (Bourgaud *et al.*, 2001). *In summa*, many of these secondary bioactive compounds can trigger toxicological or pharmacological responses in humans, and therefore are highly regarded for their nutritional value and applications in the pharma industry (anteriorly associated with ethnobotanical or phytomedicinal intergenerational knowledge), amongst other industries (Guerriero *et al.*, 2018). Thus, the interest in the production and consumption of germinated seedlings has been increasing considerably over the last decades. In this thesis, we will focus on the production of microgreens of several species with different seed coats.

3. ELECTROSTIMULATION OF PLANT TISSUE

3.1 THE USE OF ELECTRICITY IN PLANTS: A HISTORICAL PERSPECTIVE

The earliest known observation of electric phenomena can be traced back to philosopher Thales of Miletus (624–547 BC - Ancient Greece) (Figure 10).. He noticed that wool fibres were mysteriously attracted to the decorative amber of the spinner's spindles, and hypothesised it was due to amber having a "soul" which was, in reality, an electrostatic effect. To this day, this historical encounter is still remembered, as the Greek word for Amber, ἤλεκτρον, transliterated as ēlektron, is the origin of the modern word "electricity" (Lindell, 2009; Maxwell, 1873).

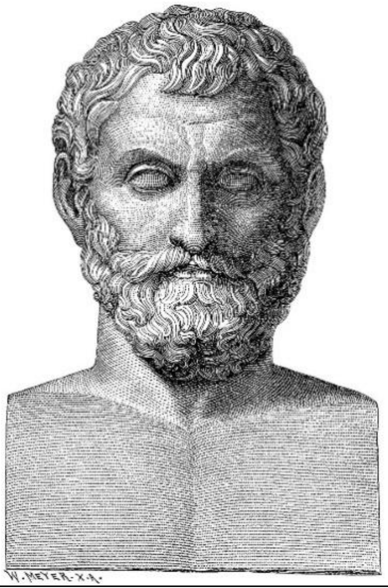


Figure 10. Thales of Miletus by Wilhelm Meyer (1875). In: *Illustrerad verldshistoria utgifven av E. Wallis. Volume I* (Public Domain).

In the 18th century, the same curiosity prevailed, and major advances in knowledge, followed by the possibility of controlling electricity, led to an increased interest in comprehending the effects of electricity on biological materials. Thus, it is believed that the 1st documented work regarding the use of electric stimuli on plants was written around 1746 by Dr. Van Maimbray of Edinburgh, after witnessing a positive impact on precocity of growth and flowering in two myrtle plants over summer, following the application of electricity over several hours a day, during an October. Several scholars obtained similar results. It is particularly relevant to the focus of this dissertation to emphasise the work of Abbé Nollet, who successfully stimulated the speed of seed germination under charged electrodes. However, these findings faced resistance from prominent scholars, such as Jan Ingenhousz, considered the father of photosynthesis, due to his contradictory results that suggested electricity hindered plant life. Finally, in 1783, Abbé Pierre Bertholon de Saint-Lazare put an end to this "schism", concluding that there was a threshold for acquiring beneficial effects (Benham, 1911). These were the pioneers of one of the most interesting applications of electricity in agriculture, according to the author's perspective, allowing discoveries such as the possibility of yield increase up to 70% of potatoes and carrots production, the acceleration of ripening of fruits and lethal *electrotrophism*, thus opening the doors to many more (Sitzmann, 2016). With this historical framework, it is possible to witness the beginning of *ElectroPriming of Seeds*.

3.2 PULSED ELECTRIC FIELDS & ELECTROPORATION

Pulsed Electric Fields are considered a non-thermal process, consisting of the application of high voltage pulses to biological materials placed between 2 electrodes (Barbosa-Canovas *et al.*, 2000), leading to a transient or permanent increase in the cell's membrane permeability. This phenomenon is widely known as *electroporation*; the term *electropermeabilization* is also employed; however, these terms are not fully interchangeable synonyms. While electroporation might be regarded as a form of cell electropermeabilization, exclusively characterised by the induction of aqueous pores in bilipidic layers, electropermeabilization is a generalized broader term that encompasses electroporation, and includes all events produced by the application of electrical fields related with the membrane permeabilization process, namely membrane channel modulation and biophysical and biochemical mechanisms (Mahnič-Kalamiza & Miklavčič, 2022; Luz *et al.*, 2021; Kotnik *et al.*, 2019). In this work, electroporation will be used to describe the method applied.

But what exactly is *electroporation*? In here, the most widely accepted theory is explained. Plasma membranes are structures that separate the cytoplasm of the cell from the external medium (or limit certain organelles, such as nucleus and vacuoles), constituted by a phospholipid bilayer that functions as a low dielectric, electrostatic barrier that can be both fluid and flexible (Robertson, 2018; Trainito, 2015; Zimmerman *et al.*, 1974; Schwan, 1957), with base electrical potential, designated by resting potential or transmembrane resting voltage (TMV) (Brosseau & Sabri, 2021; Romanenko *et al.*, 2017). This is because most cells maintain an electrical potential difference between their interior and the external medium, occurring due to the movement of different ion species, which is generated and regulated by a system of ionic or molecular pumps and diffusion channels of the plasma membrane, leading to the establishment of an electrostatic charge across the membrane (Kotnik *et al.*, 2019; Frey *et al.*, 2006; Scott, 1967). TMV is expressed by its value inside the cell, relative to the extracellular environment. In eukaryotic cells, TMV typically falls within the range of -40 to -70 mV, indicating that the intracellular potential is lower than the external one (Chrysafides *et al.*, 2023; Kotnik *et al.*, 2019). When a cell is subjected to exogenous short pulsed electric fields, an additional transmembrane potential is induced through charge movement along the electrical field lines. If that potential exceeds the critical threshold value, considered to be around 0.2-1 V, depending on membrane compressibility, permittivity, and initial thickness, the breakage of the lipidic membrane is induced due to electro-compressive forces, which will cause localised dielectric ruptures (Novac *et al.*, 2014; Ravishankar *et al.*, 2008; Toepfl, 2006) (Figure 11).

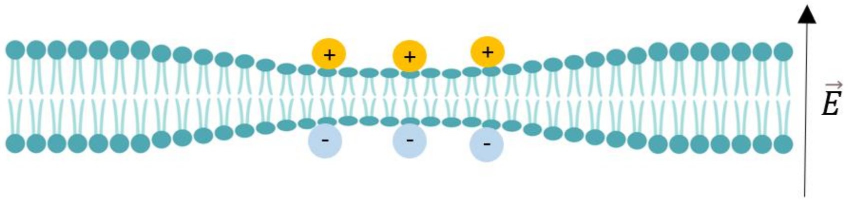


Figure 11. Representation of electro-compressive forces caused by an exogenous electrical field in a cell's bilipidic membrane of a cell (In: Aguiar-Macedo, 2024)

The first visible effect is an alteration of the membrane proteins, in particular the ones that are not anchored to the cytoskeleton, which leads to a rearrangement of the cell membrane phospholipidic bilayer structure, topology, and consistency - formation of aqueous hydrophilic pores occurs. Other phenomena also take place after the treatment, such as intracellular liquid release, solute diffusion, membrane resealing processes (in reversible PEF treatments), or even electrolysis. (Vorobiev & Lebovka, 2009).

Pores can be of transient (reversible) or permanent (irreversible) nature, considering that PEF lethality is dependent on its protocol (electrical field strength, specific energy, shape, number & duration of pulses). This protocol dependency is one of the main advantages of this technique, making it viable for distinct objectives, considering that it can both be used to increase the permeability of membranes temporarily, being the cell able to recover its homeostasis (non-lethal PEF) or permanently disrupt it, leading to the death of the cell (Sano *et al.*, 2014). Generally, researchers preconize that a critical transmembrane potential $\Delta\phi_M$ =1 V is required to guarantee the irreversible breakdown of membranes and, consequently, lead to cell death, as demonstrated by Sale and Hamilton in 1968 (Davalos *et al.*, 2005; Ho & Mittal, 1996; Sale & Hamilton, 1968). If this parameter falls below this value, electroporated cells, despite being temporary unable to control membrane permeability, caused by increased membrane conductivity and permeability to water soluble molecules that typically cannot enter the cell due to the absence of transport mechanisms, will eventually reseal the pores and fully regain their normal function, quickly establishing a new electrochemical equilibrium between intra and extracellular medium; this is known as the Gibbs-Donnan equilibrium (Waniewski *et al.*, 2021; Toepfl, 2006;).

However, cell electroporation & pore morphology (size, structure & number) depend not only on the a) chosen PEF protocol & equipment (e.g., electrical field strength (E), specific energy (w_s), pulse shape, width & number, and treatment time), but also on the b) physico-chemical characteristics of the medium (e.g. conductivity (σ), temperature and pH), c) biological properties of the cell (e.g., cell radius (R), shape and cell membrane type (bacterial) and d) the position/angle of the cell center relative to the electrical field's direction vector (θ) (Golberg *et al.*, 2016; Vorobiev & Lebovka, 2009; Ho & Mittal, 1996). To better understand this relation, we can look at the model developed by Schwan (Schwan, 1957) (Equation 1) to determine the induced TMV for single, simple, structured, spherical cells,

$$\Delta_M = 1.5ERcos\theta \qquad (1)$$

, where Δ_M is the induced TMV (V), which is directly proportional to the applied electrical field ($E\ (V/m)$) and cell radius ($R\ (rad)$), while it is possible to assess that the electroporation effect is larger when the field direction is perpendicular to the membrane ($\theta = 0^0$ and $\theta = 180^0$). Thus, Schwan was able to show that the larger the cell, the lower is the electric field needed to electroporate it; for instance, milder PEF treatments are necessary to electroporate vegetal cells, when in comparison with animal cells. Figure 12 illustrates the dependency between the Electrical Field Strength $\vec{E}$, and the cell type, shape, diameter, and disposition regarding the vector direction of the pulses.

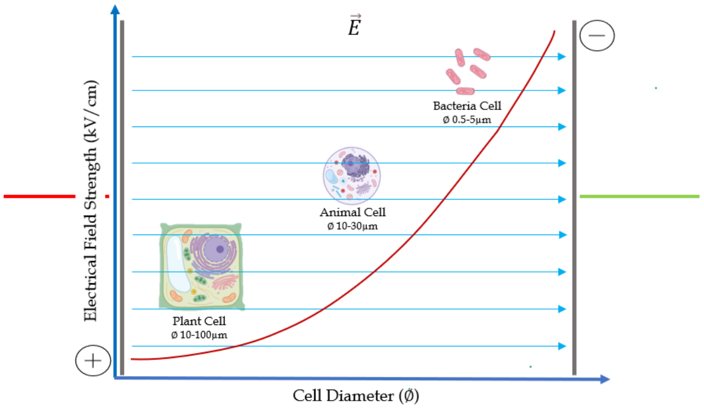


Figure 12. Graphical representation of the relationship between the required PEF treatment intensity and cell size. *In*: Aguiar-Macedo (2024).

Zimmermann & Vienken (1982) corroborate this theory; higher E values resulted not only in a larger number of pores, but also in increased pore diameter. Joshi & Schoenbach (2000) concluded that pulse width, also referred to as pulse duration, is another pivotal parameter to account for. They determined that even at higher E, PEF treatments might not be sufficient to cause irreversible electroporation if the pulse width is not long enough to allow for the growth and expansion of pores beyond the critical threshold radius. In addition, they also showed that irreversible electroporation was associated with the existence of a lower number of large pores, while reversible pores were associated with smaller diameters and higher population (Joshi & Schoenbach, 2000).

PEF can display several types of waveforms, including the most usual exponential decay, square wave, and oscillatory/sinusoidal. Square-shaped pulses are probably the most usual-shaped pulses (Barbosa-Canovas *et al.*, 2000). Electroporation, as previously mentioned, is a threshold phenomenon, requiring the maintenance of a specific E to induce pores in a plasma membrane. Square waveforms are advantageous in this regard as they can sustain the required electric field strength throughout the entire pulse. This results in reduced energy losses, a decreased risk of energy being diverted to other processes such as Joule Heating and electrolysis, and the assurance of a consistent electric field strength over extended periods. These factors contribute to higher lethality and greater energy efficiency in this waveform (Novickij *et al.*, 2022; Miklavcic & Towhidi, 2010; Saulis *et al.*, 1991). Pulses can also differ based on their polarity, classified as either bipolar or monopolar. Recently, a new interest in bipolar pulses has surfaced due to their ability to induce additional stress in the bilipidic membranes, contributing to a reduction in asymmetric membrane damage, while also improving the process from an equipment point of view, considering that it contributes to a reduction of energy consumption, a smaller risk of food electrolysis, less solid deposition on the electrodes, and reducing possible contaminations with metal ions, by diminishing corrosion of the electrodes (Novickij *et al.*, 2022; Barba *et al.*, 2015; Brito *et al.*, 2012).

3.3 SEED ELECTROPRIMING THROUGH PEF

Nowadays, given the contemporaneity of this technique, the application of electrical stimuli through Pulsed Electric Fields to seeds and their effects on growth and physicochemical characteristics are still quite limited, as demonstrated by the exhaustive review conducted by (Attri *et al.*, 2022). Published research has explored the application of Pulsed Electric Fields (PEF) with various protocols on a range of plant species, including Leaf Lettuce, Barley, Kale, Wheat, Chickpea, Mung bean, Bitter gourd, Tomato, *Medicago sativa*, Chili, Morning glory, and Green Foxtail.

In 2012, Dymek *et al.* conducted a study involving pre-sowing PEF treatments on barley seeds. The study encompassed various electrical field strengths (E) up to 1.2 kV/cm and specific energies (W_S) up to 910 J/kg. These treatments consisted of square pulses with a width of 1 ms separated by intervals of 2 s. It was concluded that PEF did not disrupt metabolic activities and processes; however, α-amylase enzyme levels were lower on PEF-treated samples, and radicle elongation was affected at 1.2kV/cm, which might have resulted from the decreased availability of sugars released from starch degradation by α-amylase.

Triticum aestivum L. (wheat) was studied by various teams relatively to its reaction to a non-lethal PEF treatment. Leong *et al.* (2016) determined that while an application of 0.5 kV/cm did not influence seed growth, a treatment of 1.4 kV/cm was able to slightly increase seedling size. However, when seeds were subjected to 2 kV/cm, a considerable reduction in coleoptile (-6 mm) and primary leaf growth (-10 mm) occurred. All pre-treated seeds showed that PEF did not interfere with nutritional properties, total phenolic content, or total vitamin C content; however, at 2 kV/cm, the proportion of reduced form Vitamin C (L-ascorbic acid) presented a twofold increase, along with a higher total glutathione content and stimulation of antioxidant enzymes (Leong *et al.*, 2016).

In another study conducted by Ahmed *et al.* in 2020, wheat seeds were subjected to up to 6 kV/cm, demonstrating that PEF can optimise water uptake (imbibition), being the best results obtained when a protocol of 6 kV/cm, 7.5 kJ/kg, and 50 pulses was applied. This process is a significant contributor to seed germination as it activates enzymes responsible for catabolizing seed reserves, such as starch, breaking these large molecules into simpler compounds like glucose, which can be used as an energy source during germination (Ahmed *et al.*, 2020; Han & Yang, 2015). In addition, Ahmed *et al.* also found that, with that PEF protocol, juice obtained from the plantlets presented an increase from 59.5% to 78.72%, which is in accordance with the results observed for plant vigour, weight, length, leaf area, nutrient intake and chlorophyll and carotenoid concentration, which itself promoted the photosynthetic process. Total phenolics were also significantly increased (Ahmed *et al.*, 2020). Thus, it is hypothesised that PEF might act as a stress, given that the damage inflicted to cell wall and protein structures leads to the opening of calcium channels and allows a faster imbibition, consequently contributing to enzymatic activity, optimizing access to growth compounds, and ultimately enhancing respiration and metabolic processes. These factors may, in turn, contribute to the accumulation of polyphenols. The increase of metabolite production as a response to stress has been reported by other researchers (Cantos *et al.*, 2000; Zhang & Björn, 2009).

Abbé Pierre Bertholon's insights were already correct in 1783, considering that most of the recently published papers confirm that, effectively, this can be viewed as a threshold phenomenon, dependent on both the PEF protocol and the physiological state of the seeds before treatment. Excessively low intensity field strengths might present no impact on seed germination, development, and biochemical characteristics; however, beyond a certain threshold, detrimental impacts become evident, leading to the inhibition of seed germination, along with significant alterations to biochemical processes. This means that, for the sake of this PEF application, we need to apply a mild PEF protocol that is strong enough to induce sublethal TMV stress and stimulate the development of microgreens by *electropriming*, while also inducing better nutritional and bioactive properties.

3.3.1 EVALUATION OF ELECTROPRIMED SEEDS GERMINATION & GROWTH

Seed germination is a complex and unique process essential for the sexual propagation of plants, ensuring genetic variability among individuals. This process serves as a key mechanism for survival and adaptation, guaranteeing the continuity of generations and the persistence of the species. It all starts when a dry seed is awakened due to water imbibition, which kickstarts the metabolic processes and stops the seed's dormancy period (Nonogaki *et al.*, 2010). Due to hydric uptake, together with other germinative-friendly conditions (i.e., temperature, O_2 presence, or specific trigger conditions, like fire, scarification, or stratification), seed cells elongate and initiate mitosis, proliferating and leading to the formation of new tissues (Talská *et al.*, 2020). Thus, the embryo develops, and a radicle protrudes on the outer layer of the seed, determining the end of the germination phase (Tuan *et al.*, 2025; Vázquez-Ramos & Sánchez, 2003). Thus, before sowing, two parameters can be determined to assess the impact of the application of Pulsed Electric Fields on the seed imbibition phase. Water Uptake, evaluated by the differences in weight after imbibition at different time stamps and the electrical conductivity (σ) of the imbibition solution, given the ability to directly relate the degree of electroporation to the increase of this parameter (Rogov & Gorbatov, 1974; Vorobiev & Lebovka, 2009).

Many factors can influence seed germination and plant emergence. Abiotic factors, such as light, temperature, humidity, seed depth, and soil characteristics, play a role just as important as biotic factors, whether intrinsic to the seed or environmentally related (Humphries *et al.*, 2018; Lamichhane *et al.*, 2018). In this particular assay, microgreens are grown in environmentally controlled conditions to reduce possible variables to a minimum, to ensure the maximum robustness of the assay.

Over plant studies, several indices were developed to assess germination, with the objective of coping with the difficulty of assessing seed germination and emergence, as a qualitative development response (Kader, 2005). One of the most relevant indices used to assess germination development is the Germination Index (GI). There are several formulas to calculate GI. In fact, GI can be used to assess information directed to individual days – Daily Germination Index (*DGI*, Equation 2) – or in a cumulative form – Cumulative Germination Index (*CGI*) (Ahmed *et al.*, 2020). While there are different variations of CGI, for this study, the index formula based on Reddy et al. (1985) and adapted by Walker-Simmons (1987) was selected. This decision was made since a maximum weight is attributed to embryos and seeds that develop earlier, at the detriment of later germinated seeds. This is supported by recent studies, such as Kader (2005) and Al-Ansari & Ksiksi (2016), which compared typically used germination indexes, concluded that CGI seemed the most comprehensive parameter as it considers both the number of germinated seeds and germination speed. This occurs since *CGI*'s used formula is as in Equation 3:

$$DGI = \frac{n_{Day} \cdot 100}{n_{total}} \tag{2}$$

$$CGI = \left(t_f \cdot n_1\right) + \left(t_{f-1} \cdot n_2\right) + \ldots + \left(1 \cdot n_f\right) \tag{3}$$

, where t_f is the last day of germination (i.e., if germination lasts 5 days, $t_f = 5$ and n is the number of seeds germinated (i.e. $n_1 =$ number of seeds germinated on day 1).

Kotowski's Coefficient of Velocity Index (*CV*) is possibly the oldest index still in use, largely due to its higher sensitivity to early germination. This happens given that CV increases when the number of germinated seedlings rises and/or when the time required for germination decreases (Brown & Mayer, 1988; Kotowski, 1927). Therefore, CV serves as an indicator of the germination velocity and can be calculated using Equation 4:

$$CV = \frac{n_{total}}{\sum(n_x . t_x)} \tag{4}$$

, where the total number of germinated seeds (n_{total}) is divided by the weighted sum of the number of germinated seeds by the day they germinated. Theoretically, the maximum possible CV is 1, which would occur if all seeds germinated on the first day (Kader, 2005).

Seed Vigour Indexes (SVI-I and SVI-II) were developed by Abdul-Baki & Anderson (1973) and comprise both the length and dry weight of the radicles or roots and shoots, and the standard germination percentage. The non-destructive approach (SVI-I) considers the radicle lengths, which are ideal to assess seed development without compromising assays. The Formula used is as demonstrated in Equation 5:

$$SVI = Germination\ Percentage \cdot Average\ Radicle\ length \tag{5}$$

Growth evaluation can also be assessed by the determination of Fresh and Dry Weight and their relation (%), Shoot and Root Length, and respective shoot-to-root ratio, and leaf area can be considered to represent the overall plant development and biomass allocation (Hilty *et al.*, 2021; Kang & Iersel, 2004). These kinds of parameters are essential to monitor the impact of different agricultural practices and to estimate species' standard growing behaviours.

3.4 PEF EQUIPMENT

The architecture of a PEF Generator is based on Pulsed Power technology, which is a science focused on the ability to store energy over a relatively long period of time - seconds to minutes - followed by its rapid release within a short time span -µs to ms -, generating large instantaneous peak powers while operating with low average input powers (Lerh & Pralhad, 2017).

A complete PEF equipment consists of both an energy storing high-voltage solid-state Marx generator (SSMG), usually including a coupled monitoring and control system, and a transducer, more commonly referred to as a treatment chamber, responsible for delivering pulses to the load placed between the electrodes (Redondo & Pereira, 2015).

Based on the required output power and PEF protocols for this assay, EPULSUS® PM1A-12, produced and commercialised by EnergyPulse Systems (Lisboa, Portugal), was selected for this study. This equipment can deliver pulses 2-200 μs almost-perfect square monopolar pulses with a maximum voltage of 12kV and pulse current of up to 250A, delivering a maximum average power of 3kW (Figure 13). Coupled to this SSMG, a static batch transducer consisting of two stainless steel electrodes encased in acrylic and housed within a safety acrylic box was selected (Figure 14).

Figure 13. Marx Generator *EPULSUS*® PM1A-12 (Courtesy of *EnergyPulse Systems*).

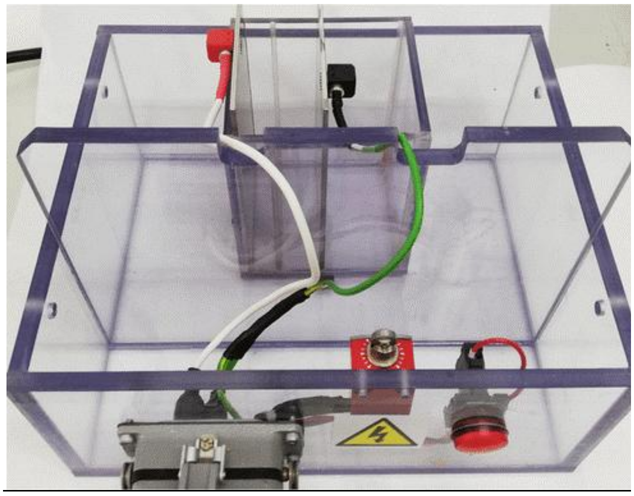


Figure 14. Batch P2P Transducer (Photo of the Author).

This treatment chamber features a parallel plate electrode (P2P) configuration with an adjustable electrode distance (d) and a maximum load capacity of 1L. This design is ideal for laboratory-scale applications, as P2P architectures provide a more homogeneous electric field distribution across the load, minimising the formation of electric field gradients (Ricci *et al.*, 2018; Zhang *et al.*, 1995).

II. COMPARATIVE STUDY OF PEF-TREATED MICROGREEN PRODUCTION

1. MATERIALS & METHODS

1.1 PLANT MATERIAL

Wild Arugula (*Arugula vesicaria* var. *sativa*), Basil (*Ocimum basilicum* L.), and Beetroot "Detroit 2" (*Beta vulgaris* subsp. v*ulgaris* var. *conditiva*) seeds were purchased (Flora Lusitana, Portugal) to perform this assay (Figure 15).

Figure 15. Beetroot, Basil, and Arugula Seeds (Photo of the Author).

The assay started by the determination of the average seed weight, by measuring the weight of 10 seeds of each species, to aid with the determination of the weight necessary to accomplish a desired seed rate. Seed density was established based on the literature review previously mentioned, on several calculation online tools, and, ultimately and most importantly, in visual assessment of their size and the empirical expertise of a local microgreen producer (Volodymyr Goncharuk, MicroEcoGrow, Viseu). The final seed density and respective seed weight per replicate tray, with 333 cm^2 each, are presented in Table 2.

Table 2. Average Seed Weight.

	Average Seed Weight (g)	**Seed Density (n° seeds/cm^2)**	**Seed Weight per 333 cm^2 (g)**
Arugula	0.000228 ± 0.00038	12	0.9133
Basil	0.00183 ± 0.00055	5	3.0567
Beetroot	0.01427 ± 0.00338	1.75	8.3242

Initially, seed processing started by weighing the determined volume of seeds for each replicate, and each species was separated into 3 replicates for each group of subjects - Control, PEFA (2 kV/cm, PEFB (3 kV/cm, PEFC (4 kV/cm).

Additionally, after PEF treatment, 10 seeds were counted and separately sown in substrate-filled petri dishes for each replicate, with each seed being numbered. These seeds were used to assess germination and radicle length evaluation during the first 5 days after sowing, by visual assessment of germination events and the determination of the radicle length (a seed was considered successfully germinated when a radicle with ≥1 mm was measured), together with photographic registration (Figure 16).

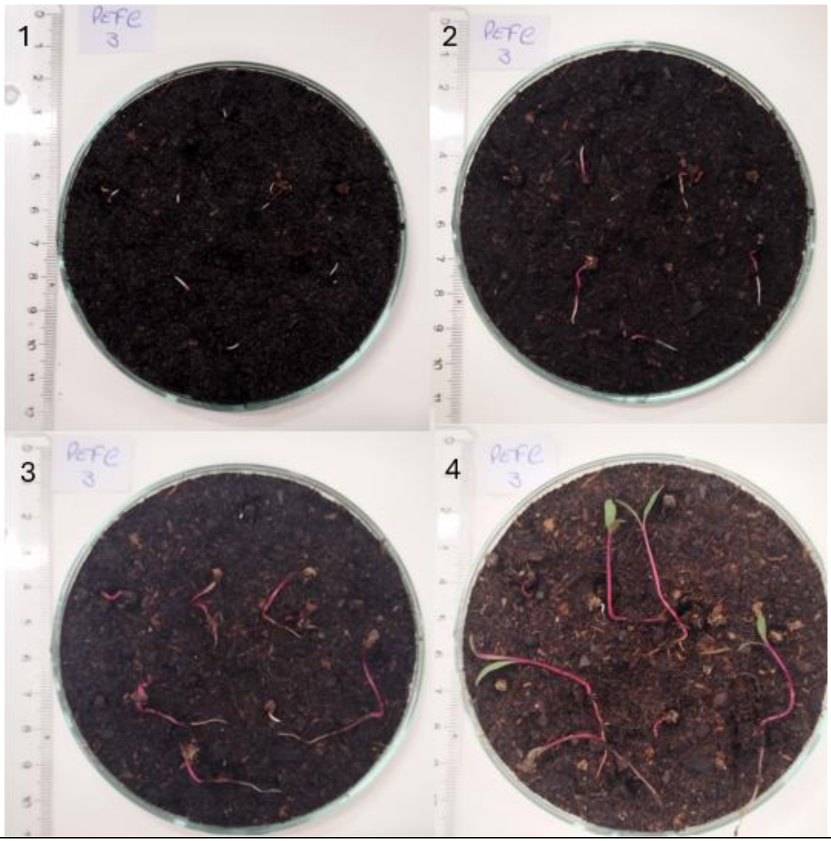


Figure 16. *Beta vulgaris* L. Example of the Progression of Germination and Radicle Elongation at different growth stages. 1 – 2 days After Sowing; 2- 3 days after Sowing; 3 – 5 days after Sowing; 4 – 9 days after Sowing (Photo of the Author).

1.2 PEF EQUIPMENT, PROTOCOLS & APPLICATION

Before advancing with this assay, extensive research on the few published and the author's unpublished information was conducted to determine the most viable protocols to at least avoid the crossing of the threshold responsible for separating the seed stimulation from seed inactivation. After consideration, the protocols for this assay were selected. Beetroot seeds were treated and sown on 21st February, while Arugula and Basil were processed on the 22nd.

PEF application was made resorting to an EPULSUS® PM1A-12 (EnergyPulse Systems, Lisbon, Portugal). This equipment delivers positive square-shaped pulses with a maximum capacity of 12 kV/250 A and 3 kW average output power. Seeds were subjected to PEF with a parallel design, plate-to-plate (P2P) static treatment chamber. While the two electrodes could be placed in three positions, to modulate the distance between them (d). The selected gap for these assays was fixed in $d = 2cm.$ This is based on the direct relation with the applied pulse voltage amplitude (U_p) - usually represented in kV – allowing the calculation of the amplitude of the Electrical Field (E, in kV/cm) of the treatment, by following formula (Equation 5):

$$E = \frac{U_p}{d} \tag{5}$$

Thus, for each type of treatment, a different pulse voltage was applied, 4 kV for PEFA, 6 kV for PEFB and 8 kV for PEFC, to obtain the desired E for each protocol.

To calculate the Specific Energy (W_s) applied, usually given in kJ/kgIt is necessary to realize intermediate steps to determine the necessary parameters. For such, it is necessary to have knowledge not only of pulse voltage, but also current amplitude (I) and the number of applied pulses (n_p). In our assays, n_p was equal in all assays, where 10 pulses were applied at a frequency $f = 1Hz$, with a pulse width (τ) of 10µs. Pulse Current (I_p) is an output value, obtained as demonstrated in Equations 3 and 4 (page 36 & 37), where A_c is the electrode area and σ denotes the electrical conductivity of the sample under treatment, as per Ohm's Law (Equations 6 and 7, Figure 17, 18, and 19):

$$I_p = \frac{U_p}{R} \text{ and } R = \frac{d}{Ac} \cdot \frac{1}{\sigma} \tag{6}$$

$$I_p = \frac{U_p \cdot \sigma \cdot Ac}{d} \tag{7}$$

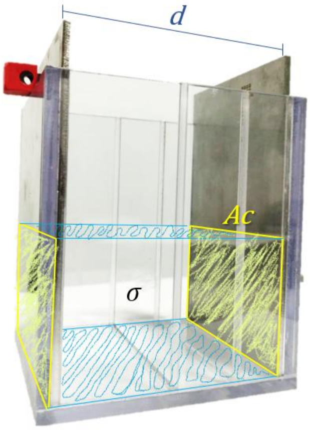


Figure 17. Representation of Ohm's Law parameters (*In*: Aguiar-Macedo (2024).

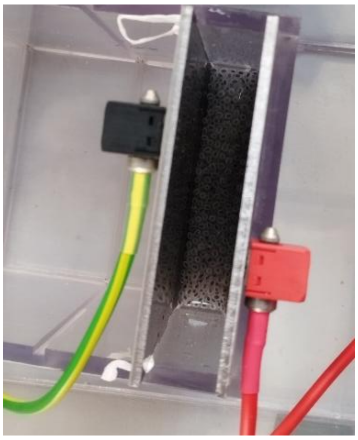


Figure 18. Basil Seeds in the Treatment Chamber, top view (Photo of the Author).

Figure 19. Basil Seeds in the Treatment Chamber, lateral view (Photo of the Author).

In these assays, $A_c = 30cm^2$ for beetroot and basil, and $A_c = 25cm^2$ for arugula. With this information, the total energy applied (W_t), in Joules, and the treatment time (t_{on}), referring to treatment duration (Equations 8 and 9).

$$t_{on} = \tau \cdot n_p \tag{8}$$

$$W_t = U_p \cdot I \cdot t_{on} \tag{9}$$

Now, with all the necessary parameters, W_s can be determined based on Equation 10, as applied by Aguiar-Macedo *et al.*, 2023 and Barros *et al.*, 2022:

$$W_s = \frac{W_t}{m} \tag{10}$$

, where m is the adimensional mass of the product under study. Being the volume of the sample placed in the treatment chamber 50mL for arugula and 60mL for basil and beetroot, and estimating a density of $\rho = 1kg/L$, it is possible to directly extrapolate these values to kg.

A resume of the PEF protocols applied is demonstrated in the following table (Table 3).

Table 3. PEF Protocol Parameters for each subject assay of arugula, basil, and beetroot.

Species	Subject	E (kV/cm)	I (A)	Calculated σ (based on A) (mS/cm)	Energy per Pulse *(J)*	TC Volume (mL)	W_s (kJ/kg)
Arugula	Control	-	-	-	-	50	-
	PEFA	2	1-2	0.03	0.1		0.020
	PEFB	3	4-5	0.06	0.3		0.060
	PEFC	4	9	0.1	0.6		0.120
Basil	Control	-	-	-	-	60	-
	PEFA	2	7-8	0.13	0.3		0.050
	PEFB	3	15-16	0.18	1		0.167
	PEFC	4	25	0.21	2		0.333
Beetroot	Control	-	-	-	-	60	-
	PEFA	2	25	0.41	1		0.167
	PEFB	3	97-100	1.08	5.9		0.983
	PEFC	4	144-145	1.2	11.5		1.917

NOTE: *n*= 3. For current amplitudes between 1 and 25A an associated error of 20% in measurement must be considered.

During treatment, two interesting observations were made. At higher Current (I) values, each pulse became audible; and when basil seeds were treated with PEF, seed aroma was perceived as more fragrant, which can be an empirical indicator of a successful electroporation, leading to the release of aromatic compounds.

1.3 GERMINATION, GROWTH, AND HARVEST CONDITIONS

Four perforated aluminium trays with 20x50cm were previously prepared with ± 560g of germination substrate $(h = \pm 2\ cm)$ (Siro Germinação Bio) with characteristics compatible with the ones mentioned in *2.2.2. Growing Medium & Trays* (Table 4). 450 mL of distilled water were added per tray to guarantee an optimal environment for germination. Each tray was divided into three equal areas (20 cm x 16.67cm), with the objective of using each section for a replicate of the same treatment (Figure 20).

Table 4. Characteristics of the Growing Medium provided by the supplier (Siro Germinação Bio).

	Soil Characteristics
pH	5.5-6.5
Electrical Conductivity (σ)	150 µS/cm
Granulometry	0-8 mm
Organic Matter	>60%
Composition	*Sphagnum* blonde peat, Pine bark humus, coco peat, and organic biological fertilizer of animal origin NPK 9-3-3 (2.0 g/L).

Figure 20. DIY Perforated Aluminium Microgreen Tray (20x50cm) used for assays (Photo of the Author).

After seed weighting, PEF application, and the imbibition process with the respective assessment, seeds were spread evenly within each tray section according to their treatment and replicate number. Trays were covered and stacked to create a germination-friendly environment, free of light and highly humid. In addition, they were placed in an environmental simulation plant growth chamber (FitoClima 1200, Aralab, Lisbon, Portugal), with programmable temperature, relative humidity, airflow, and photoperiod (Figure 21).

Figure 21. Aralab FitoClima 1200 (*In:* aralab.pt)

The selected program is explained in Table 5:

Table 5. FitoClima Programmed Growing Environment.

Parameter	Selection
Temperature (°C)	23
Relative Humidity (%)	50
Airflow (%)	50
Photoperiod	During the first 5–6 days after sowing, the photoperiod was set to zero to ensure complete blackout . On February 27th, it was adjusted to a 12-hour light/12-hour dark cycle.

Germination and growth were monitored daily, from sowing to harvest. Each replicate was irrigated with 50 mL of distilled water per day, while seeds allocated to petri dishes received 10 mL per day.

1.4 ANALYTICAL ASSESSMENT

1.4.1 GERMINATION AND PLANT GROWTH EVALUATION

The impact of PEF priming on seed imbibition can be assessed using three parameters, as previously mentioned. Water Uptake was evaluated by the weight difference before and after imbibition, comparing this between groups, and by the electrical conductivity (σ) of the imbibition solution (Vorobiev & Lebovka, 2009; Rogov & Gorbatov, 1974). In theory, electroporation should lead to an increase in water uptake and electrical conductivity of the medium, due to the opening of pores that allow a greater influx of water to the intracellular space, along with the release of ions in the opposite direction. Temperature is also monitored to ensure and determine if there is a differential that could affect the robustness of the assay. While other motives could be considered, either the monitoring of the temperature increase by the energy input from PEF application or by the start of physiological and biochemical reactions, such as enzyme activation and the beginning of cellular metabolism – a phenomenon known as imbibition heat – these are completely disregarded as they are negligible and would require a highly sensible apparatus capable of detecting minimal differences (Rodrigues *et al.*, 2022; Domergue *et al.*, 2019)

The evaluation of germination was performed throughout the assay, allowing the calculation of some indices commonly used to assess plant development. These parameters are based on the daily observation of germination events (where ≥ 1mm is considered germinated) and/or the measurement of the respective shoot length. The selected indices for this study were the Daily Germination Index (DGI), Cumulative Germination Index (CGI) (Walker-Simmons, 1987; Reddy *et al.,* 1985), and Kotowski's Coefficient of Velocity Index (*CV*). They were already explained in section *3.3.1. Evaluation of ElectroPrimed Seeds Germination & Growth* (page 35).

1.4.2 POST-HARVEST: NUTRITIONAL & PHYSICO-CHEMICAL ANALYSIS

1.4.2.1 Microgreen Extracts Preparation

Considering that one of the objectives of this dissertation is to assess physicochemical parameters, bioactive compounds, namely Chlorophyll *a* and *b*, Total Phenolic Content, and Antioxidant Capacity (ABTS & DPPH), it is essential to find an extract preparation protocol compatible with the extraction of a variety of compounds. This is particularly important, considering the different characteristics of these compounds. For instance, while dietary Chlorophylls *a* and *b* are mainly considered as lipophilic pigments (Ferruzzi & Blakeslee, 2007), most plant phenolics possess a hydrophilic nature (Arzola-Rodríguez *et al.*, 2022). In addition, Chlorophylls are also very susceptible to degradation by heat, light, and acidic conditions (Villaño *et al.*, 2016).

Thus, to lead to process optimisation, it was necessary to select one or two extract protocols capable of obtaining multi-use effective plant extracts.

Ethanol–water solvents seem to be the most suitable for the extraction of bioactive compounds, as their different polarities complement themselves. In addition, the possibility of using them in various proportions and their acceptability for human consumption makes it to pose as an ideal solvent system. Several studies corroborate that a 70% hydroethanolic solution is the ideal equilibrium for recovering the highest percentage of vegetal phenolic content, while also extracting and preserving the maximum concentration of bioactive compounds with antioxidant capacity. For instance, Popa *et al.* (2023) compared three extracts of *Helichrysum arenarium* inflorescences: aqueous, 70% ethanol, 80% methanol, and analysed various parameters, amongst with there were DPPH and Total Phenolic Content, obtaining optimum extraction with 70% hydroethanolic solvent. In 2021, Silva *et al.* studied the effect of the solvent choice on the extraction of polyphenols and bioactive compounds with antioxidant potential in *Euterpe edulis* (*açai juraça*) and reached similar conclusions: for the various methods of analysis (i.e., HPLC, FRAP, DPPH) used, 70% ethanol solvent presented better than pure ethanol or pure water (Silva *et al.*, 2021). Thus, two extraction protocols were selected. Both used a 70/30 ethanol-water solution, with a sample-to-solvent ratio of 1:10. The key difference laid in the extraction method: one was subjected to magnetic stirring at 700 rpm (Variomag® Multipoint HP, Daytona Beach, Florida, USA) while the other underwent ultrasonic treatment at 37kHz, resorting to an S60 H Elmasonic ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany). The extraction time and temperature were the same for both – 30 min at 20 °C. Afterwards, the samples were subjected to filtration using Whatman n° 3 paper filter and a vacuum pump, being this process finalised by the refrigeration of the samples, protected from light, until analysis. This distinction between aiding extraction technologies not only contributes to the main objective of this thesis but also engages in a side quest, which allows us to determine which technique might be the most suitable.

For the assessment of Chlorophyll *a*, Chlorophyll *b* & Total Carotenoids, exceptionally, a different extract was prepared based on the methodology of Lichtenthaler (1987). These pigments are liposoluble and can be extracted from fresh living plant material by organic solvents (Lichtenthaler, 1987). Thus, the protocol consisted of the collection of healthy microgreens' leaves, followed by their weighing and joining at a ratio of 1:10 to Diethyl Ether (Fisher Scientific, Loughborough, United Kingdom). This was followed by grinding, using a stainless-steel pestle and mortar. The resulting "pulp" was separated from the liquid by filtration. Immediately after processing, spectroscopical analysis took place to avoid the degradation of chlorophylls, reducing its detrimental impact on the results. After analysis, the remaining sample was protected from light by being wrapped in aluminium foil and refrigerated.

1.4.3 UV-VIS SPECTROPHOTOMETRIC ANALYSIS

1.4.3.1 Chlorophyll *a*, Chlorophyll *b* & Total Carotenoid

To determine if there were any differences regarding the development of pigment compounds amongst treatments, the two main chlorophylls present in higher plants (vascular) - *a* and *b* - and total carotenoids were measured using Lichtenthaler (1987) methodology. Leaf pigments were assessed by spectrophotometric analysis (UV-1280 Spectrophotometer, Shimadzu, Kyoto, Japan), by direct measurements of the diethyl ether extract absorbance at $\lambda = 470nm$, $\lambda = 642.2nm$ and $\lambda = 660.6nm$. The respective readings were applied in the following formulas (Equations 11-14):

$$C_a = 10.05A_{660.6} - 0.97A_{642.2} \tag{11}$$

$$C_b = 16.36A_{642.2} - 2.43A_{660.6} \tag{12}$$

$$C_{a+b} = 7.62A_{660.6} + 15.39A_{642.2} \tag{13}$$

$$C_{x+c} = \frac{1000A_{470} - 1.43C_a - 35.87C_b}{205} \tag{14}$$

, where C_a refers to Chlorophyll *a*, C_b to Chlorophyll *b*, C_{a+b} to Total Chlorophylls and C_{x+c} to Total Carotenoids, in µg/mL of plant extract (Lichtenthaler, 1987).

1.4.3.2 Total Phenolic Content

Total polyphenolic content (TPC) was analysed based on spectrophotometric analysis, using the Folin-Ciocalteau method (Singleton *et al.*, 1999). Initially, a calibration curve was prepared using gallic acid as a standard ($y = 3.6214x + 0.1881$; $R^2 = 0.9961$). The determination of TPC was based on an optimised Singleton & Rossi methodology and performed as described by Dulyanska (Dulyanska *et al.*, 2022b; Santos *et al.*, 2014; Singleton & Rossi, 1965). This method was chosen for its viability, cost-effectiveness, and environmental sustainability, as it requires a minimal amount of sample and reagents while eliminating the need for prolonged high-temperature incubation, leading to energy savings. Succinctly, it consists of the addition of 125µL of the extract sample, 125 µL of Folin-Ciocalteau reagent, and 750 µL of Mili-Q water to a vial. The mixture was incubated in the dark for 6 minutes, followed by the addition of 2 mL of 5% Sodium Carbonate (Na_2CO_3). The solution was then vigorously shaken to ensure proper homogenization. The tubes were protected from light and left to react for 60 minutes. After this period, the absorbance of the standards was measured at $\lambda = 750\ nm$. A blank sample was prepared by replacing the extract with 125 µL of Milli-Q water, and an auto-zero adjustment was performed using Milli-Q water before sample readings. The results are expressed in mg of Gallic Acid per g of Fresh Weight (mg GAE/g FW).

1.4.4 ANTIOXIDANT CAPACITY

Phenolic compounds, vitamin C and E, and Carotenoids are the main constituents of the enormous group of bioactive compounds present in vegetable dietary sources with antioxidant capacity (Bengtsson & Hagen, 2008; Lindsay & Astley, 2002). Total Antioxidant Capacity can be evaluated by several methods based on the reduction of radicals by hydrogen atom transfer (i.e., FRAP method) and single electron transfer (DPPH and ABTS assays) (Boskou, 2015). To ensure more robust results, two different assays were chosen for analysis of the microgreen extracts: DPPH and ABTS radical scavenging assays.

1.4.4.1 DPPH Scavenging Assay

DPPH method consists of the determination of the reduction of free radicals by the donation of the antioxidant's hydrogen atoms. Thus, the higher the reduction of free radicals, the higher the antioxidant capacity of the product under study. The following protocols were described by Dulyanska *et al.*, (2022), being adapted from Brand-Williams (Brand-Williams *et al.*, 1995). In the 1st assessment, 2,2-diphenyl-1-picrylhydrazyl hydrate radical - DPPH, in short, was used by preparation of an ethanolic solution ($6x10^5$ M), which was immediately protected from light. Blank reading was performed at $\lambda = 517\ nm$ with ethanol, to avoid its interference with the measurements. This was followed by the adjustment of DPPH solution to an Abs ≈ 0.700 (Abs_0), to ensure a standardised starting reading for reliable results. Next, 100µL of the sample, together with 2 mL of the DPPH solution, were collected into a test tube, shaken, and left to react for 30 minutes, protected from light. The absorbance of the prepared sample is then read at $\lambda = 517\ nm$ (Abs_{sample}). The absorbance is adapted to the previously prepared calibration curve ($y = -0.2933x + 0.6395$; $R^2 = 0.995$) with Trolox and the results were given in % of DPPH inhibition (Equation 11), and in µg Trolox Equivalents per gram of Fresh Weight (µg TE/g FW).

$$\%\,Inhibition = \frac{Abs_0 - Abs_{sample}}{Abs_0 * 100} \qquad (11)$$

1.4.4.2 ABTS Scavenging Assay

The evaluation of the antioxidant capacity through ABTS (Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) methodology is performed thanks to the reagent's ability to oxidise and produce a stable free cationic radical $ABTS^{\bullet+}$. The process began with the preparation of a calibration based on standardised Trolox solutions ($y = -0.2117 + 0.5905;\ R^2 = 0.999$). The selected protocol requires, as 1st step, the addition of 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) to the ABTS radical aqueous solution (7 mmol/L) (Pellegrini *et al.*, 2003; Re *et al.*, 1999). This will turn an otherwise colourless solution into a green-blue toned one. ABTS solution is incubated at room temperature for 12-16h, achieving oxidative stability. Finalised this period, the solution is diluted in ethanol at a rate of 1:80, which is followed by the determination of the solution's absorbance at $\lambda = 734\,nm$ (autozero) and being adjusted if necessary (Abs ≈ 0.700). The microgreen extracts preparation consisted of its addition (0.1 mL) to 2.0 mL of the $ABTS^{\bullet+}$ solution, followed by homogenization and a resting period of 15 minutes to allow chemical reactions to take place (Dulyanska *et al.*, 2022a). In the end, the absorbance at $\lambda = 734\,nm$ was determined, with the results being expressed in % ABTS inhibition and µg TE/g FW.

1.4.5 TOTAL SOLUBLE SOLIDS

Total Soluble Solids, TSS, expressed in °Brix, were measured using a digital refractometer (95200-001, Alla France, Chemillé-en-Anjou, France). This method estimates the concentration of TSS, constituted mainly by sugars, but not exclusively, including other refractive compounds present in the sample, in this case, the extract, such as organic acids (Paul *et al.*, 2010).

The procedure was quite simple, as it consisted of, at first, calibrating the equipment with distilled water, and then proceeding with the direct measurement of the °Brix of each of the hydroalcoholic (70/30) extracts previously produced.

1.4.6 NEAR INFRARED REFLECTANCE SPECTROSCOPY

FT-NIR, short for Fourier Transform Near-Infrared Reflectance, was the selected spectroscopy technique to quantify the nutritional profile of the obtained microgreens. These analyses were performed using a NIRMaster™ (BÜCHI Labortechnik, Flawil, Switzerland). This equipment is equipped with product-specific internal calibration curves and can assess several nutritional parameters, as well as relevant amino acids, including Humidity, Fat, Protein, Crude Fiber, Ash, Starch, NDF (neutral detergent fibre), Lysine, Cystine, Methionine, and Phosphorus. The results are presented as percentages of dry matter.

The sample processing followed this protocol: the remaining volume of freshly harvested microgreens, after sensory analysis and extract preparation, was weighed and placed in an oven at 65°C for 12 hours. This protocol was selected based on a preliminary baby green drying assay, conducted to determine the necessary drying time for this type of product at a conservative temperature, minimising potential degradation due to heat. After this period, dried microgreens were pulverised to assess dry weight, using a stainless-steel pestle and mortar. The final sample was collected into a vial and used to perform the readings.

1.4.7 POST-HARVEST: SENSORY ANALYSIS

During the conception of this experiment, it was determined that the final part of this study should focus on assessing possible organoleptic differences caused by seed *electropriming* through PEF. This is particularly important given that microgreens are typically consumed fresh, making it essential to, at the very least, maintain the quality of the final product. Therefore, this analysis is crucial to identifying any potential negative sensory alterations that could impact the viability of PEF as a pre-treatment. Considering the prime objective, a descriptive test type was selected, as it is based on the perception of specific attributes and their respective intensities (Lawless & Heymann, 2010; Sidel & Stone, 2006), capable of providing analytical and reliable information (Armstrong, 1999). Thus, this approach is an ideal candidate to detect possible differences and, consequently, evaluate the impact of the treatments on different microgreen species.

A descriptive sensory test was performed based on the guidelines of the Quantitative Descriptive Analysis (QDA) methodology developed by Sidel & Stone. QDA requires the evaluation of the relative intensity of multiple descriptors using a continuous scale, conducted by a trained tasting panel of 5 to 15 panellists specialised in the food product under study and its respective sensory attributes (Sidel & Stone, 2006; Stone *et al.*, 2004).

However, since microgreens are a niche product, finding a pre-trained panel was challenging. As a result, we were unable to fully adhere to this QDA assumption and instead adapted this method and relied on panellists with experience in the sensory analysis of other food products (i.e., wine & dairy).

Sensory descriptive attributes of microgreens have not been well described in the literature, thus, the selected parameters to apply in this study are mainly based on previous microgreen sensory analysis. However, to the best of our knowledge, the existing published studies are mainly focused on consumer preference and acceptability (Bafumo *et al.*, 2024; Komeroski *et al.*, 2024; Dhaka *et al.*, 2023; Tan *et al.*, 2020; Di Gioia *et al.*, 2017b; Xiao *et al.*, 2015). Therefore, these studies tend to demonstrate a tendency in assessing the acceptability of Texture, Taste, Odour, and Visual Appearance in a general and broader term, with few evaluating specific sensory attributes. Studies by Caracciolo (2020) and Michell (2020) considered parameters such as *Sweetness*, *Aroma*, *Astringency*, *Bitterness*, *Texture,* and *Heat*.

To give robustness to the attribute's selection, bibliography regarding sensory attributes of similar agriproducts, namely leafy vegetables, was also briefly analysed and used to help adjust to our objective. Talavera-Bianchi, Chambers & Chambers (2010) developed an attribute lexicon for leafy greens' flavour and concluded that attributes such as *Bitter*, *Astringent*, *Heat/Burn*, and *Sweet*, which align with Caracciolo (2020) and Michell (2020). Dhakal *et al.* (2021) considered the attributes *Fibrous* and *Crispiness* as essential to assess Texture in a comparative study between organic and conventionally produced leafy greens. Zhou *et al.* (2025) conducted a sensory analysis of leafy greens treated with ultrasounds for post-harvest microbial control. The visual component was assessed based on the greenness of the samples to determine whether there were any signs of deterioration or browning (Zhou *et al.*, 2025).

Based on these studies, the final categorised attribute descriptors were selected and are outlined in Table 6:

Table 6. List of Selected Sensory Descriptors.

Descriptors			
Visual	Mouthfeel & Flavour		Aroma
Integrity	Bitter	Fibrous	Intensity
Colour Intensity	Hot	Moist	
Tonality	Crispy	Astringent	
	Sweet	Aftertaste	

All sensory attributes were assessed using a Likert-type ordinal numerical scale from 1 to 10 (Likert, 1932). A score of 1 indicated that the attribute was undetected/weak, while a score of 10 indicated that it was easily detected/excellent. Additionally, an extra parameter – Global Evaluation – was included, also rated on a scale of 1 to 10. The sensory evaluation sheet provided to each panel member can be found in Appendix I.

1.4.7.1 Tasting Panel

The tasting panel consisted of 9 panellists, including 6 women and 3 men, with ages ranging from 23 to 59 years. The selected panel was composed of ESAV academics, researchers and students, all from the field of Food Technology and/or with knowledge in sensory evaluation of other food categories.

1.4.7.2 Sample Preparation & Tasting Sessions

To ensure an unbiased evaluation during the sensory analysis, the microgreens were coded with random three-digit codes prior to the tasting to prevent panellists from recognising the samples, which could otherwise influence their perceptions (Ray, 2021). These are discriminated in Table 7.

Table 7. Sensory Sample Coding.

Species	Treatment	Sample Code
Beetroot	Control	518
	PEFA	169
	PEFB	652
	PEFC	434
Arugula	Control	926
	PEFA	372
	PEFB	163
	PEFC	524
Basil	Control	275
	PEFA	786
	PEFB	192
	PEFC	842

Similarly to leafy vegetables, microgreens can see their quality altered during storage, given chlorophyll breakdown and respiration, which leads to dehydration (Agüero *et al.*, 2008). Microgreens are highly perishable, and storage conditions such as temperature, humidity and packaging material are crucial factors to ensure a longer shelf-life. Typically, it is expected that microgreens last around 2-3 days when stored at room temperature, but this can be extended up to 7-14 days at 10 °C and 12-14 days at 4.44 °C (Sharma *et al.*, 2023; Sun *et al.*, 2015). In fact, freshly cut leafy greens, which fall under the category "Time and Temperature Control for Safety", are required by the Food and Drug Administration (FDA) to be stored at a temperature no higher than 5 °C (FDA, 2009). Lastly, Komeroski *et al.* (2024) documented the loss of quality in microgreens over 10 days, with Chlorophyll content and Colour being significantly affected after 3 days of storage. This not only impacts the overall quality and sensory attributes of the produce and, therefore, their sensory assessment but also leads to a diminishment of the health benefits provided by the consumption of microgreens (Komeroski *et al.*, 2024).

With this in mind, it was decided to prepare the sample with the same methodology presented by Michell *et al.* (2020). Microgreens were kept in the germination chamber at 23 °C, and were harvested only 1h before tasting, being washed and refrigerated at 4 °C until consumption. Even quantities of the 3 replicates were selected to prepare a representative sample of each treatment code. Because of different leaf sizes, the amount served to panellists was different depending on species. 10 minutes before tasting, they were placed in coded glass containers and exposed to room temperature. Tasting samples were presented to panellists as observed in Figure 22.

Figure 22. Sensory Analysis Preparation.

Tasting sessions took place in one of ESAV's laboratories, late in the afternoon, in a comfortable and distraction-free environment to reduce interferences that could affect the accuracy of the results. At the beginning of the tasting session, each participant was instructed about each attribute and to cleanse their palates between species with water and unsalted crackers (Caracciolo *et al.*, 2020; Michell *et al.*, 2020; Talavera-Bianchi *et al.*, 2010).

After verifying all individual sensory evaluation data, the scores were recorded in Excel® for further analysis.

1.5 DATA ANALYSIS

All analyses were performed in triplicate in randomized block design, with data collected during both the germination and development phases of the microgreens, as well as at the end of the cycle, culminating in the harvest and holistic analysis of each microgreen produced. The collected data for each parameter are presented as Mean ± Standard Deviation (SD). All data were subjected to statistical analysis using IBM SPSS Statistics, Version 28.0.1.0 (142) (SPSS Inc., Chicago, IL, USA). A significance level of $p = 0.05$ was applied in all statistical analyses.

Depending on the case, different statistical tests were employed. To evaluate the assumptions of homogeneity of variance-covariance matrices, Levene's and M-Box tests were performed (Marôco, 2011; Hair *et al.*, 2009). For example, Analysis of Variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) were employed to assess statistical differences amongst physicochemical parameters and sensory analysis, respectively. If statistically significant differences were found, *post hoc* Tukey and Bonferroni tests were performed to detect the differences between treatments. In addition, Partial Eta Square (η_p^2) is also reported, as it is considered quite relevant, considering it quantifies the effect size, indicating the proportion of differences between subjects explained by the factor under study, which is, in this case, the PEFpriming treatment. For example, an η_p^2 value of 0.60 can be interpreted as the treatment factor being able to explain 60% of the assessed differences amongst subjects. Marôco (2011) defined value outlines where η_p^2 can be classified as small (< 0.05), medium (≤ 0.25), large (≤ 0.5), and very large (> 0.5).

For growth evaluation, the statistical test selection fell on a Linear Mixed Model.

Graphics presented in this dissertation were generated using GraphPad Prism, Version 8.0.2 (GraphPad Software Inc, San Diego, CA, USA) and Microsoft® Excel®, for Microsoft 365, version 2312, Build 16.0.17126.20132 (Microsoft Corporation, Redmond, WA, USA).

2. RESULTS & DISCUSSION

2.1 GERMINATION & PLANT GROWTH EVALUATION

2.1.1 WATER UPTAKE & ELECTRICAL CONDUCTIVITY

The results obtained from the assessment of seed imbibition, possibly modulated by electroporation due to PEF application, were monitored through the evaluation of water uptake, electrical conductivity and temperature.

The results obtained for Beetroot seeds are summarised in Table 8:

Table 8. Evaluation of Water Uptake, Electrical Conductivity during Beetroot Seed Imbibition.

		Parameter	Control	PEFA	PEFB	PEFC	*p*	η_p^2
Beetroot	Before Imbibition	Dry Weight (g)	8.3224^{b} ± 0.0046	8.3215^{b} ± 0.0042	8.3251^{b} ± 0.0024	8.3264^{a} ± 0.0028	0.022	0.255
		σ H_2O (mS/cm)	0.09^{a} ± 0.00	0.08^{bc} ± 0.01	0.09^{ab} ± 0.01	0.08^{c} ± 0.00	<0.001	0.556
		°C	13.9^{a} ± 0.1	13.9^{b} ± 0.0	13.9^{b} ± 0.0	13.9^{b} ± 0.0	0.016	0.273
	1h after Imbibition (10min before PEF)	σ (mS/cm)	1.57^{a} ± 0.05	1.54^{b} ± 0.02	1.44^{b} ± 0.17	1.58^{a} ± 0.01	0.047	0.287
		°C	13.9 ± 0.1	13.9 ± 0.0	13.9 ± 0.0	13.9 ± 0.1	0.064	0.200
	Post-PEF	σ (mS/cm)	2.54 ± 0.06	2.56 ± 0.07	2.58 ± 0.08	2.50 ± 0.08	0.182	0.139
		°C	15.4^{b} ± 0.1	15.5^{a} ± 0.1	15.4^{b} ± 0.1	15.5^{a} ± 0.1	<0.001	0.634
	4h Post-PEF (Before Sowing)	Weight (g)	12.1583^{b} ± 0.0945	12.5353^{ab} ± 0.3407	12.5591^{a} ± 0.0912	12.3299^{a} ± 0.0924	<0.001	0.459
		σ (mS/cm)	3.05^{b} ± 0.10	3.19^{a} ± 0.07	3.18^{a} ± 0.08	3.16^{b} ± 0.09	0.007	0.312
		°C	18.6^{a} ± 0.2	18.1^{b} ± 0.1	18.2^{b} ± 0.1	18.1^{b} ± 0.1	<0.001	0.777

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. $n=9$. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

Before imbibition, the goal was to ensure the most consistent initial conditions across all groups to eliminate any potential differences that could influence the impact of the treatment on seed imbibition. Tap water was used to kickstart seed imbibition. Before imbibition σ and temperature of the tap water were determined. Although all three parameters showed statistically significant differences between groups, these differences can be considered negligible from a practical standpoint, given the minimal variations between groups and the very low standard deviations. 1h after imbibition and 10 minutes before PEF application, it is possible to see that the seeds were subjected to similar temperature conditions; in addition, we can also see a slight σ difference, considered statistically significant, which we can attribute to the variability rates of seed imbibition. This difference was diluted immediately post-PEF treatment. 4h after PEF-treatment, seeds were separated from water, dried with a paper towel and weighed.

PEF-treatment significantly affected the water uptake capacity of seeds in relation to control for protocols PEFB and PEFC, with 3 and 4 kV/cm, respectively, given that the weight was significantly higher when in comparison with control subjects. The η_p^2 value indicates a large effect, with 45.9% of the variability attributed to the PEF treatment. As expected, σ was higher for all treated subjects, indicating that electroporation was successful, to different degrees, as it indicates the release of ionic content to the imbibition solution.

The results for Arugula species are summarised in Table 9:

Table 9. Evaluation of Water Uptake, Electrical Conductivity during Arugula Seed Imbibition.

		Parameter	Control	PEFA	PEFB	PEFC	p	η_p^2
Arugula	Before Imbibition	Dry Weight (g)	0.9134 ± 0.0004	0.9134 ± 0.0002	0.9136 ± 0.0002	0.9135 ± 0.0004	0.565	0.061
		σ H_2O (mS/cm)	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.09 ± 0.00	0.100	0.333
		°C	14.0^{ab} ± 0.1	13.8^{c} ± 0.1	14.0^{b} ± 0.1	13.9^{bc} ± 0.0	<0.001	0.625
	1h after Imbibition (Immediately before PEF)	σ (mS/cm)	0.17^{a} ± 0.01	0.16^{b} ± 0.01	0.16^{b} ± 0.01	0.17^{ab} ± 0.09	<0.001	0.333
		°C	14.4^{a} ± 0.1	14.5^{b} ± 0.1	14.6^{b} ± 0.0	14.7^{c} ± 0.1	<0.001	0.704
	Post-PEF	σ (mS/cm)	0.19^{b} ± 0.01	0.23^{a} ± 0.02	0.22^{a} ± 0.00	0.23^{a} ± 0.01	<0.001	0.727
		°C	15.4^{c} ± 0.1	16.2^{b} ± 0.3	16.6^{a} ± 0.2	16.8^{a} ± 0.1	<0.001	0.917
	4h Post-PEF (Before Sowing)	Weight (g)	-	-	-	-	-	-
		σ (mS/cm)	0.30^{b} ± 0.01	0.32^{a} ± 0.02	0.30^{b} ± 0.00	0.31^{b} ± 0.01	0.004	0.331
		°C	18.1 ± 0.1	18.1 ± 0.2	18.0 ± 0.2	17.9 ± 0.2	0.071	0.195

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. $n=9$. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

The imbibition process evaluation for arugula presented some challenges stemming from the seed morphology. Arugula seeds have a reduced size, which hinders the capacity of drying the excess water of the seeds with a paper towel before the final weighing, given that many seeds can be lost in the process. Consequently, for this species, weight was only measured prior to imbibition, while the electroporation effect was monitored by σ evolution. Once again, the initial conditions are found to be identical to all groups, considering significant differences were not found for Initial Dry Weight ($F_{(3.32)}$=0.689, p=0.565), nor for the imbibition water electrical conductivity ($F_{(3.32)}$=5.333, p=0.100). Immediately Post-PEF treatment, differences in terms of σ

became evident ($F_{(3.32)}=28.467$, $p=0.100$, $\eta_p^2=0.727$), where PEFA and PEFC exhibited an increase of conductivity 21% superior to Control, being this difference attributed to the variable treatment by 72.7%. However, this difference diminished over time. At the 4-hour mark, although significant differences were still observed, only PEFA exhibited them. This suggests that, over time, the diffused ions transferred from the seeds to the solution reached the same equilibrium between Control, PEFB and PEFC.

Table 10 presents the results obtained for the evaluation of imbibition parameters for Basil:

Table 10. Evaluation of Water Uptake, Electrical Conductivity during Basil seed imbibition.

		Parameter	Control	PEFA	PEFB	PEFC	P	η_p^2
Basil	Before Imbibition	Dry Weight (g)	3.0560 ± 0.0003	3.0559 ± 0.0003	3.0562 ± 0.0005	3.0562 ± 0.0005	0.212	0.129
		σ H_2O (mS/cm)	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.01	0.327	0.273
		°C	14.4^{a} ± 0.0	14.3^{b} ± 0.1	14.3^{b} ± 0.1	14.4^{ab} ± 0.1	0.007	0.314
	1h after Imbibition (Immediately before PEF)	σ (mS/cm)	0.28^{a} ± 0.01	0.24^{b} ± 0.02	0.23^{b} ± 0.02	0.27^{a} ± 0.02	<0.001	0.634
		°C	15.7 ± 0.1	15.8 ± 0.2	15.8 ± 0.1	15.7 ± 0.1	0.05	0.220
	Post-PEF	σ (mS/cm)	0.35^{c} ± 0.01	0.34^{c} ± 0.01	0.37^{b} ± 0.00	0.38^{a} ± 0.01	<0.001	0.926
		°C	15.3^{c} ± 0.1	15.6^{a} ± 0.1	15.3^{c} ± 0.1	15.5^{b} ± 0.1	<0.001	0.824
	4h Post-PEF (Before Sowing)	Weight (g)	42.4782^{b} ± 0.6799	41.5625^{b} ± 1.1760	43.0547^{a} ± 1.2489	43.6487^{a} ± 0.9732	<0.001	0.38
		σ (mS/cm)	0.40^{c} ± 0.01	0.41^{b} ± 0.01	0.41^{b} ± 0.01	0.43^{a} ± 0.01	<0.001	0.746
		°C	17.4 ± 0.1	17.5 ± 0.2	17.4 ± 0.1	17.3 ± 0.1	0.109	0.170

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. *n=9.* PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

Basil's initial samples and conditions statistics for initial weight and water conductivity were homogeneous (Weight: $F_{(3.32)}=1.585$, $p=0.212$; Water Conductivity: $F_{(3.32)}=1.238$, $p=0.327$). Immediately before PEF treatment, two groups were formed: Control and PEFC presented significantly higher σ than PEFA and PEFB. Despite this, after treatment, the highest σ values group switched to PEFB and PEFC, presenting significantly higher σ in comparison to Control and PEFA ($F_{(3.32)}=132,889$, $p<0.001$, $\eta_p^2=0.926$), being 92.6% of this difference attributed to the treatment effect. 4h post-PEF, weight and σ were significantly higher in seeds treated with protocols PEFB and PEFC, which indicates that water imbibition was modulated by electroporation of the seed tissues.

While analysis denotes that statistically, there were significant temperature differences, these can be discarded, as the differences are very small, which renders them as irrelevant in this context.

The evaluation of water uptake and electrical conductivity during the imbibition of basil, beetroot, and arugula seeds under different PEF treatments revealed several trends and species-specific responses. For instance, the magnitude of the change in membrane permeability varied between species as observed by the different behaviour observed by σ and Water Uptake. While Basil presented the highest increase in σ and Final Seed Weight for PEFC, σ was not altered for Beetroot immediately post-PEF treatment. For Beetroot, differences were only found 4h post-PEF, with PEFB presenting the highest increase in Final Seed Weight, which contrasts with σ values, considering the highest was obtained for protocol PEFA. In opposition, Arugula presented the largest difference in σ immediately post-PEF (PEFA and PEFC); however, after 4h, PEFA was the only group significantly higher.

Some studies corroborate these results, while others contradict this, which displays the complexity of the protocol selection. For instance, a treatment of 3 kV/cm, when accompanied by a specific energy of 19.8 kJ/kg, represented an increase in water uptake of 25% in wheat seeds before malting; however, at a lower energy protocol (9.9 kJ/ kg), a decrease in moisture absorption was observed (Polachini *et al.*, 2023). In turn, Ahmed *et al.* (2020) reported that wheat seeds subjected to PEF pre-treatment at an electric field strength below 6 kV/cm (100 μs pulse width, 25 pulses) did not exhibit increased water uptake. However, when treated at 6 kV/cm with 50 pulses, the seeds showed a significant increase in water content, rising from 50.93% to 56.56%, suggesting that both field strength and specific energy play a critical role in modulating water absorption during early seed hydration.

These results indicate that PEF effects are species-dependent, possibly due to differences in seed coat structure and cell membrane composition. Thus, for each species, protocol optimisation must be tailored. PEF can contribute to a rapid hydration of the seeds, which accelerates activation of amylase and protease activity, allowing for an earlier availability of seed reserves to aid germination.

2.1.2 GERMINATION INDEXES & RADICLE LENGTH

Results obtained for radicle length and the number of germinated seeds for beetroot, arugula, and basil over five days under different treatment conditions are summarised in Table 11.

Table 11. Radicle length (mm) and number of germinated seeds.

		Day									
		1	n_1	2	n_2	3	n_3	4	n_4	5	n_5
Beetroot	Control	4.29 ± 2.84	12	6.66 ± 5.18	16	12.08 ± 9.04	18	18.86 ± 12.28	18	21.87 ± 13.70	19
	PEFA	4.16 ± 3.17	16	7.47 ± 5.53	16	13.40 ± 11.56	16	18.59 ± 16.95	16	19.53 ± 17.06	16
	PEFB	5.44 ± 3.91	16	8.53 ± 7.17	17	15.59 ± 12.65	17	19.92 ± 17.11	18	21.28 ± 18.07	18
	PEFC	5.00 ± 3.16	20	9.64 ± 5.49	21	20.30 ± 12.87	22	26.89 ± 16.29	23	29.09 ± 16.13	23
Arugula	Control	1.97 ± 1.75	18	7.35 ± 5.67	23	15.04 ± 9.01	24	20.23 ± 11.09	24	22.06 ± 11.22	25
	PEFA	3.45 ± 3.70	20	10.89 ± 8.43	23	19.21 ± 11.41	24	21.06 ± 12.79	25	22.58 ± 12.99	25
	PEFB	4.73 ± 3.45	22	12.82 ± 7.30	25	20.43 ± 11.42	29	24.72 ± 12.14	29	29.95 ± 13.70	29
	PEFC	3.97 ± 3.49	19	10.77 ± 7.46	24	20.04 ± 10.61	26	23.89 ± 10.05	26	26.32 ± 9.61	28

Table 11. Radicle length (mm) and number of germinated seeds.

		Day									
		1	n_1	2	n_2	3	n_3	4	n_4	5	n_5
Basil	Control	1.50 ± 0.71	5	3.09 ± 1.77	11	4.64 ± 2.98	14	7.12 ± 4.23	14	7.63 ± 4.62	15
	PEFA	1.44 ± 1.33	9	2.13 ± 1.38	16	3.32 ± 2.07	17	4.08 ± 3.86	19	4.92 ± 4.43	19
	PEFB	2.46 ± 2.9	12	4.29 ± 3.94	14	6.83 ± 5.26	15	8.97 ± 6.79	15	10.93 ± 9.03	15
	PEFC	2.15 ± 3.02	13	3.28 ± 1.76	16	6.91 ± 5.26	17	8.09 ± 5.78	17	9.27 ± 5.64	17

NOTE: n_t = number of seeds germinated on Day t. $n_{total} = 30$ seeds. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

A Linear Mixed Model (LMM) was conducted to examine the effects of the Day, Treatment and their interaction, concluding that, for Beetroot, a significant main effect associated to Treatment was found ($F_{(3.338)}$=4.575, *p*=0.004), meaning that, statistically, it is possible to consider that treatments did influence radicle length. The same was observed for Arugula - ($F_{(3,468)}$ = 6.188, *p*<0.001) and for Basil - ($F_{(3,268)}$ =8.292, *p*<0.001). For all species, differences regarding both radicle length and number of germinated seeds per subject were clear from day 1. Besides that, observing the table implied that treatment effects were consistent over time, for all species, which means the results were not diluted throughout the assay. This is corroborated by statistical analysis, considering the Interaction Day x Treatment was not significant for all species (Beetroot: $F_{(12,338)}$ = 0.482, *p*=0.925 Arugula: $F_{(12,468)}$ = 0.399, *p*=0.964; Basil: $F_{(12,268)}$=0.594, *p*=0.847).

In addition, with the information collected over the 1st five days of germination, growing indices were determined. Daily Germination Index (*DGI*), Cumulative Germination Index (*CGI*), for Kotowski's Coefficient of Velocity Index (*CV*), and Seed Vigour Index (*SVI*), are resumed in Table 12.

Table 12. Results of the Germination and Growth Indexes.

					DGI				
		CGI	*CV*	*SVI*	n_1	n_2	n_3	n_4	n_5
Beetroot	Control	233	0.613	875	40.0	53.3	60.0	60.0	63.3
	PEFA	240	1.000	1042	53.3	53.3	53.3	53.3	53.3
	PEFB	253	0.818	1135	53.3	56.7	56.7	60.0	60.0
	PEFC	319	0.793	1939	66.7	70.0	73.3	76.7	76.7
Arugula	Control	327	0.694	1324	60.0	76.7	80.0	80.0	83.3
	PEFA	339	0.758	1505	66.7	76.7	80.0	83.3	83.3
	PEFB	384	0.725	2196	73.3	83.3	96.7	96.7	96.7
	PEFC	349	0.622	1667	63.3	80.0	86.7	86.7	93.3
Basil	Control	154	0.484	127	16.7	36.7	46.7	46.7	50.0
	PEFA	217	0.559	148	30.0	53.3	56.7	63.3	63.3
	PEFB	206	0.789	437	40.0	46.7	50.0	50.0	50.0
	PEFC	231	0.773	402	43.3	53.3	56.7	56.7	56.7

NOTE: n_t = number of seeds germinated on Day t. $n_{total} = 30$ seeds. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm. *CGI*: Cumulative Germination Index; *CV*: Kotowski's Coefficient of Velocity; *SVI*: Seed Vigour Index; *DGI*: Daily Germination Index.

The effect of PEF treatments is evident for all species, with the optimal protocol varying for each species. For instance, while the best results were observed with the 4 kV/cm protocol (PEFC) for Basil and Beetroot, a 3 kV/cm protocol (PEFB) seemed to be the most suitable for Arugula. This conclusion is supported by both the average radicle length and the number of seeds germinated, further corroborated by the Germination Indexes.

For Beetroot, all germination indices were highest when a PEFC protocol was applied, except for *CV*, which is more sensitive to early germination. The peak CV value was observed in seeds treated with the PEFA protocol.

For Basil, while DGI was consistently higher in PEF-treated groups, the variability in CV suggests that the impact of PEF treatment on DGI was not entirely consistent over time. For example, the highest DGI on day 1 was for PEFC, but by Day 5, it was attributed to seeds treated with a protocol of 2 kV/cm (PEFA).

For Arugula, the highest CGI and SVI were recorded for PEFB-treated groups, reinforcing the idea that 3 kV/cm was the most effective treatment for this species.

With respect to Kotowski's Coefficient of Velocity (CV), it is noteworthy that all PEF treated seeds presented a higher CV in comparison with control, with the exception of Arugula treated with the most intense electrical field strength. PEF treatment clearly improved radicle length and germination rates and CV.

These results support once again the hypothesis of a species-specific PEF effect, given the differences. The observed improvements in germination and radicle length under certain conditions can be beneficial in agricultural applications to enhance seedling establishment.

2.2 POST-HARVEST: NUTRITIONAL & PHYSICO-CHEMICAL RESULTS

2.2.1 UV-VIS SPECTROPHOTOMETRIC ANALYSIS

2.2.1.1 Chlorophyll a, Chlorophyll b & Total Carotenoid

The effect of the different PEF treatments on Chlorophyll a (C_a), Chlorophyll b (C_b), Total Chlorophylls (C_{a+b}), and Carotenoids are resumed in Table 13.

Table 13. Chlorophyll *a*, Chlorophyll *b*, Total Chlorophylls & Carotenoids, after harvest, in µg/mL of extract**.**

	Parameter	**Control**	**PEFA**	**PEFB**	**PEFC**	***P***	η_p^2
Beetroot	*Chlorophyll a*	$11.68^{a} \pm 0.56$	$4.74^{b} \pm 0.48$	$9.91^{ab} \pm 0.188$	$9.82^{ab} \pm 2.18$	0.033	0.237
	Chlorophyll b	$4.74^{a} \pm 0.48$	$3.38^{ab} \pm 1.48$	$3.17^{b} \pm 1.56$	$2.73^{b} \pm 0.93$	0.008	0.306
	Total Chlorophyll	$16.42^{a} \pm 0.76$	$12.87^{b} \pm 2.66$	$13.08^{ab} \pm 3.36$	$12.55^{b} \pm 2.98$	0.013	0.284
	Carotenoids	$4.47^{a} \pm 0.33$	$3.55^{b} \pm 0.59$	$3.86^{ab} \pm 0.66$	$4.00^{ab} \pm 0.80$	0.030	0.241
Arugula	*Chlorophyll a*	$12.36^{a} \pm 1.93$	$9.08^{b} \pm 0.56$	$6.17^{c} \pm 1.51$	$6.77^{c} \pm 0.32$	<0.001	0.805
	Chlorophyll b	$3.54^{a} \pm 1.15$	$3.22^{a} \pm 0.57$	$0.65^{b} \pm 0.56$	$1.49^{b} \pm 0.66$	<0.001	0.731
	Total Chlorophyll	$15.91^{a} \pm 3.06$	$12.31^{b} \pm 0.76$	$6.81^{c} \pm 2.05$	$8.26^{c} \pm 0.67$	<0.001	0.797
	Carotenoids	$5.06^{a} \pm 0.76$	$3.82^{b} \pm 0.33$	$2.88^{c} \pm 0.63$	$2.96^{c} \pm 0.12$	<0.001	0.759
Basil	*Chlorophyll a*	$14.07^{ab} \pm 2.92$	$11.96^{b} \pm 2.75$	$15.64^{a} \pm 2.22$	$16.81^{a} \pm 1.53$	0.001	0.389

Table 13. Chlorophyll *a*, Chlorophyll *b*, Total Chlorophylls & Carotenoids, after harvest, in µg/mL of extract**.**

Parameter	Control	PEFA	PEFB	PEFC	*P*	η_p^2
Chlorophyll b	$4.02^{bc} \pm 2.82$	$2.47^{c} \pm 0.97$	$5.51^{ab} \pm 2.33$	$6.86^{a} \pm 2.26$	0.001	0.384
Total Chlorophyll	$18.09^{bc} \pm 5.64$	$14.43^{c} \pm 2.43$	$21.15^{ab} \pm 4.55$	$23.66^{a} \pm 3.75$	<0.001	0.426
Carotenoids	$5.90^{b} \pm 1.64$	$5.55^{b} \pm 0.98$	$6.58^{ab} \pm 1.00$	$7.73^{a} \pm 0.94$	0.002	0.361

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

For Beetroot and Arugula, PEF treatments led to a significant reduction of C_a, C_b, C_{a+b}, and Carotenoids. However, for Beetroot, the effect size of the treatment was considered relatively low, with a maximum of 30.6% of the variability attributed to PEF. In contrast, for arugula, the treatment had a large effect size with a minimum η_p^2=0.731, meaning that at least 73.1% of the differences could be attributed to PEF application.

Basil contradicted this trend. While PEFA also resulted in a decrease in chlorophyll and carotenoid concentrations, more intense protocols (PEFB and PEFC, at 3 and 4 kV/cm, respectively) led to an increase in all pigment concentrations: C_a, C_b, C_{a+b}, and Carotenoids. However, in this case, the statistical effect of the treatment was lower, with a maximum of 42.6% of the variability attributed to PEF.

In summa, pigments were significantly affected by the application of PEF, exhibiting decreased levels of chlorophylls and carotenoids, with the exception of Basil species. PEF significantly affected pigment concentrations, generally leading to a decrease in chlorophylls and carotenoids. For Arugula, the most affected group was PEFB, which is an interesting observation, as this same group also exhibited the most significant increase in radicle length and CGI. This suggests that the reduction in pigment concentrations may be associated with a greater dispersion of these compounds throughout the plant biomass, potentially due to enhanced tissue development resulting from the treatment.

2.2.1.2 Total Phenolic Content

In order to better understand the impact of PEF treatments across species and extraction methods, multivariate analyses were conducted under two perspectives: i) globally, by species, to assess possible differences between the selected extraction methods, and ii) separately, by species and extraction type, to determine the impact of each PEF treatment on the antioxidative content, Total Phenolic Contend, and Total Soluble Solids of each extract,

On the i) approach, it was possible to conclude that the extraction methodology presents considerable effects on the concentration of compounds within the extracts:

Effect: Extraction Method:

- Beetroot: $F_{(5, 60)}$=5.084, p<0.001, η_p^2=0.770
- Arugula: $F_{(5, 60)}$=4.777, p<0.001, η_p^2=0.285
- Basil: $F_{(5, 60)}$=12.109, p<0.001, η_p^2=0.502

Interaction: Extraction Method x Treatment

- Beetroot: $F_{(15, 186)}$=3.081, p<0.001, η_p^2=0.321
- Arugula: $F_{(15, 186)}$=2.182, p=0.008, η_p^2=0.150
- Basil: $F_{(15, 186)}$=2.657, p=0.001, η_p^2=0.176

Therefore, it is possible to conclude that the type of extraction methodology – Magnetic Stirring and Ultrasound -significantly affected the extraction capacity of the compounds under study, as it was observed by the results obtained by effect analysis of both *Extraction Method* and the interaction of *Extraction Methodology x Treatment*. This is easily corroborated by the data tables displayed over the course of this subchapter, where, generally speaking, higher extraction rates are found in extracts subjected to Ultrasound treatment, when compared with Magnetic Stirring.

In addition, across all species, in a general way, it is viable to conclude that all PEF treatments present a strong and significant antioxidant activity of the plant extracts, considering that, for the condition Treatment, MANOVA's results show:

Effect: Treatment:

- Beetroot: $F_{(15,77.697)}=34.498$, $p<0.001$, $\eta_p{}^2=0.429$
- Arugula: $F_{(15,77.697)}=11.045$, $p<0.001$, $\eta_p{}^2=0.441$
- Basil: $F_{(15,77.697)}=13.136$, $p<0.001$, $\eta_p{}^2=0.514$

Thus, it was viable to advance with the ii) approach, to allow for the detection of differences caused by the different PEF protocols applied.

Starting with the comparison between Total Phenolic Content (TPC), it was possible to find some key differences between its concentration on microgreens subjected to different PEF-priming protocols. The results for TPC are shown in Figure 23.

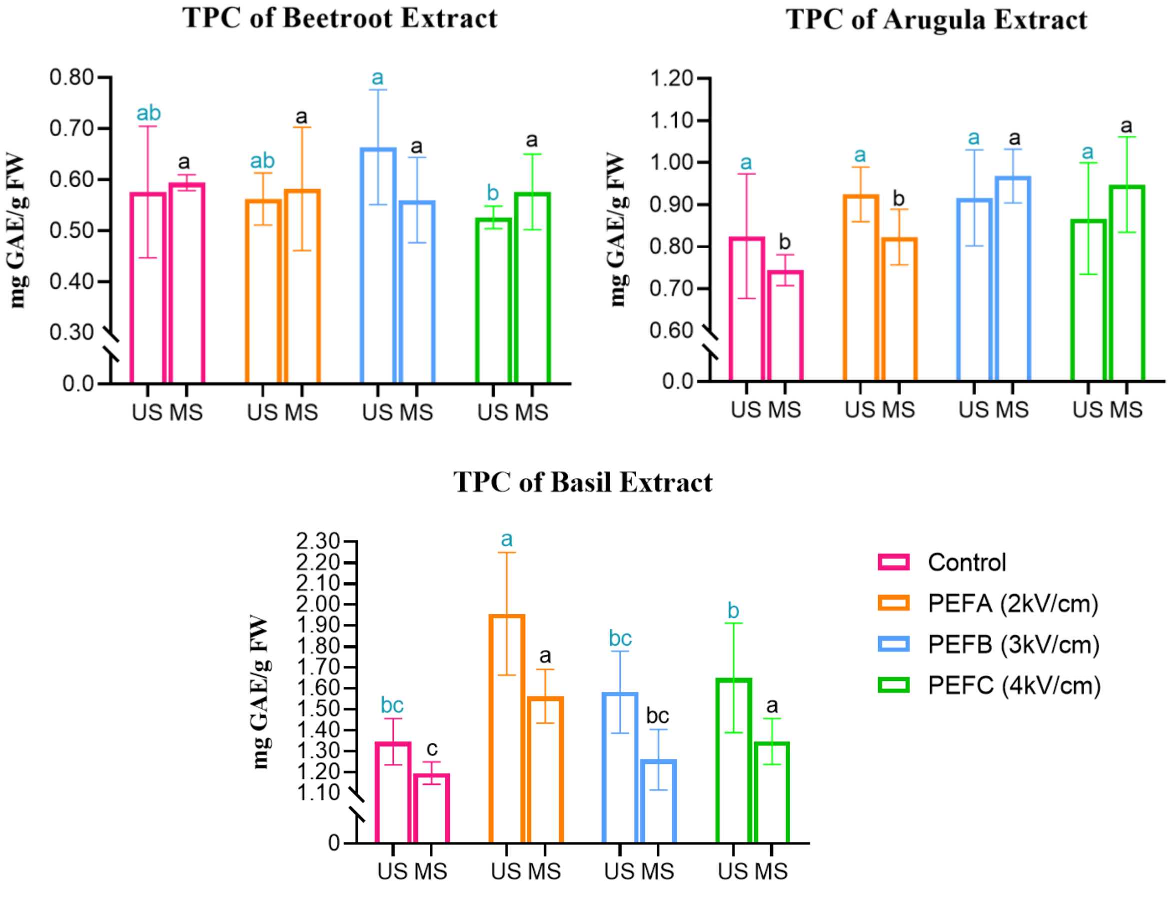


Figure 23. Total Phenolic Content in Beetroot, Arugula and Basil Microgreens. Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. Statistical comparisons were conducted within each colour group. MS: Magnetic Stirring, US: Ultrasound.

Regarding Beetroot, it is possible to observe that, for extracts obtained by Magnetic Stirring (MS), there were no significant differences observed between all subjects. However, for the same species, but in extracts obtained with the aid of Ultrasound, PEFB (3 kV/cm) presented a significantly higher concentration of TPC, representing a 15% increase in TPC when compared to the Control. For Arugula, the opposite trend is observed, with no statistically significant differences between subjects obtained with US extraction methodology. In contrast, for MS extraction, a significant increase of up to 30% concentration of TPC was obtained for more intense PEF treatments, PEFB and PEFC protocols.

At last, for Basil, all protocols represented an increase in the TPC on both extract types. The highest TPC concentration was obtained with US extraction, when it increased 45%, from 1.345 to 1.956 mg GAE/g FW, with a protocol of 1 kV/cm. Regarding MS extraction, the peak was also observed for PEFA protocol, which allowed for an increase from 1.196 to 1.562 mg GAE/g FW, representing 30.5% more TPC in this extract.

2.2.1.3 Antioxidant Capacity

The results of the DPPH and ABTS assays are presented by species in the following tables. Antioxidant capacity is expressed both as a percentage of inhibition and as micrograms of Trolox equivalents per gram of fresh weight (µg TE/g FW).

The data for Beetroot is shown in Table 14. Consistent with the trends observed for TPC, US proved more effective for the recovery of bioactive compounds from microgreens. In this species, the most effective seed priming protocols for enhancing the antioxidant potential were those involving higher electric field intensities, 3 to 4 kV/cm. Statistically significant increases in antioxidant capacity, as measured by DPPH, were observed in PEF-treated samples, ranging from 10.8% (PEFB, US) to 12.4% (PEFC, MS) inhibition increases when compared to the respective controls.

A similar pattern was found with the ABTS assay, where all PEF-primed samples exhibited significantly higher antioxidant capacities than the controls. This reinforces the conclusion that seed priming with more intensive pulsed electric fields can effectively enhance the synthesis or accumulation of antioxidant compounds in beetroot microgreens.

Table 14. Antioxidant Capacity (DPPH and ABTS) of Beetroot.

	Parameter	**Extract**		**Control**	**PEFA**	**PEFB**	**PEFC**	***p***	η_p^2
Beetroot	*DPPH*	*US*	% inhibition*	$42.82^{c} \pm 1.29$	$44.12^{bc} \pm 1.52$	$46.35^{a} \pm 0.11$	$44.37^{b} \pm 0.63$	<0.001	0.623
			µg TE/g FW	$7.96^{c} \pm 0.31$	$8.28^{bc} \pm 0.37$	$8.82^{a} \pm 0.03$	$8.34^{b} \pm 0.15$		
		MS	% inhibition*	$39.81^{b} \pm 1.71$	$37.95^{b} \pm 2.66$	$38.86^{b} \pm 1.28$	$44.73^{a} \pm 0.59$	<0.001	0.720
			µg TE/g FW	$7.24^{b} \pm 0.41$	$6.78^{b} \pm 0.64$	$7.00^{b} \pm 0.31$	$8.43^{a} \pm 0.14$		
	ABTS	*US*	% inhibition*	$57.29^{b} \pm 13.18$	$70.20^{a} \pm 7.49$	$59.14^{b} \pm 2.83$	$69.41^{a} \pm 1.77$	0.001	0.389
			µg TE/g FW	$67.85^{b} \pm 22.09$	$89.50^{a} \pm 12.56$	$70.94^{b} \pm 4.75$	$88.16^{a} \pm 2.96$		
		MS	% inhibition*	$36.87^{b} \pm 13.37$	$67.06^{a} \pm 12.94$	$65.27^{a} \pm 11.60$	$58.17^{a} \pm 8.77$	<0.001	0.623
			µg TE/g FW	$33.61^{b} \pm 22.41$	$84.22^{a} \pm 21.70$	$81.23^{a} \pm 19.46$	$69.32^{a} \pm 27.84$		

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. *% inhibition obtained from 1:5 diluted fresh weight plant extract. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

The results for Arugula are presented in Table 15.

Table 15. Antioxidant Capacity (DPPH and ABTS) of Arugula.

	Parameter	Extract		Control	PEFA	PEFB	PEFC	p	η_p^2
Arugula	DPPH	US	% inhibition	70.19^{ab} ± 4.15	68.37^{ab} ± 5.48	62.96^{b} ± 8.38	73.19^{a} ± 6.83	0.015	0.275
			µg TE/g FW	14.59^{ab} ± 1.00	14.15^{ab} ± 1.33	12.84^{b} ± 2.03	15.32^{a} ± 1.65		
		MS	% inhibition*	70.47^{ab} ± 4.57	68.37^{ab} ± 5.48	62.96^{b} ± 8.38	73.19^{a} ± 6.83	0.015	0.274
			µg TE/g FW	14.65^{ab} ± 1.11	14.15^{ab} ± 1.33	12.84^{b} ± 2.03	15.32^{a} ± 1.65		
	ABTS	US	% inhibition	29.48 ± 2.81	31.17 ± 8.20	29.91 ± 0.90	31.52 ± 3.89	0.796	0.034
			µg TE/g FW	21.22 ± 4.70	24.06 ± 13.75	21.93 ± 1.51	24.63 ± 6.52		
		MS	% inhibition*	39.74^{ab} ± 11.69	28.54^{c} ± 5.43	30.28^{bc} ± 3.99	40.68^{a} ± 7.58	0.002	0.358
			µg TE/g FW	38.42^{ab} ± 19.60	19.65^{c} ± 9.10	22.56^{bc} ± 6.70	39.40^{a} ± 12.71		

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. *% inhibition obtained from 1:5 diluted fresh weight plant extract. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

In contrast to what was observed for beetroot, no clear advantage was detected for US extraction over SM in enhancing antioxidant capacity, as both extracts exhibited similar trends. The highest DPPH inhibition values were obtained with PEFC treatments in both extracts, indicating that the most intense electric field protocol might enhance the antioxidant potential of this species. Specifically, for US extracts, microgreens whose seeds were subjected to PEFC exhibited a statistically significant increase in DPPH inhibition (+5% vs Control), suggesting that electric field strength plays a crucial role in modulating antioxidant capacity ($\eta_p^2 = 0.275$). A similar pattern was observed for SM extracts, reinforcing this trend.

Regarding ABTS, no statistically significant differences were found among US extracts (p=0.796), suggesting that this method may not be sensitive enough to detect variations in antioxidant capacity in arugula under these extraction conditions. However, for AM extracts, a significant effect was observed (p=0.002; $\eta_p^2 = 0.358$), with PEFC treatment again yielding the highest inhibition percentage (40.68%), significantly surpassing the control and PEFA. These results suggest that for Arugula, the ABTS method is more responsive to differences in seed priming treatments when SM is used, and that PEFC can be the most suitable to enhance the extraction of ABTS-reactive compounds.

Basil DPPH and ABTS results are displayed in Table 16.

Table 16. Antioxidant Capacity (DPPH and ABTS) of Basil.

	Parameter	Extract		Control	PEFA	PEFB	PEFC	p	η_p^2
Basil	DPPH	US	% inhibition*	79.44^{b} ± 1.36	78.76^{b} ± 0.39	79.22^{b} ± 0.68	81.25^{a} ± 1.07	<0.001	0.527
			µg TE/g FW	16.83^{b} ± 0.33	16.67^{b} ± 0.09	16.78^{b} ± 0.17	17.27^{a} ± 0.26		
		MS	% inhibition*	79.44^{b} ± 1.36	78.76^{b} ± 0.39	79.22^{b} ± 0.68	81.25^{a} ± 1.07	<0.001	0.527
			µg TE/g FW	16.83^{b} ± 0.33	16.67^{b} ± 0.09	16.78^{b} ± 0.17	17.27^{a} ± 0.26		

Table 16. Antioxidant Capacity (DPPH and ABTS) of Basil.

Parameter	Extract		Control	PEFA	PEFB	PEFC	p	η_p^2
ABTS	*US*	% inhibition*	28.95 ± 3.14	30.47 ± 1.27	31.25 ± 4.62	27.39 ± 2.42	0.060	0.204
		µg TE/g FW	20.33 ± 5.26	22.88 ± 2.13	24.19 ± 7.74	17.71 ± 4.06		
	MS	% inhibition*	30.64 ± 5.00	28.31 ± 5.10	31.53 ± 2.67	28.34 ± 1.14	0.204	0.132
		µg TE/g FW	23.17 ± 8.39	19.26 ± 8.55	24.66 ± 4.47	19.31 ± 1.91		

NOTE: Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. *% inhibition obtained from 1:5 diluted fresh weight plant extract. PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

Overall, DPPH inhibition and concentration values were high across all subjects, which demonstrates the antioxidant potential of this species. While no statistically significant differences were observed among treatments in US extracts, when using MS, however, PEFC treatment presented a statistically significant enhancement in antioxidant activity with a large effect size of 52.7% attributed to the PEF treatment ($p<0.001$; $\eta_p^2 = 0.527$). However, realistically, this only represents an increase of 2.6% of antioxidant capacity.

Contrary to what was observed in the DPPH assay, results were not significantly different between treatments in any of the extraction modalities. This might indicate one of two conclusions: there is a less pronounced impact of PEF priming on ABTS-reactive compounds under this extraction method, or there was no influence of the treatment on antioxidant activity. Considering the results obtained for DPPH, the 1st hypothesis might be the correct one, as there is the possibility that each assay - DPPH and ABTS - might target different antioxidant compounds.

Based on the present results, PEF treatments, particularly the PEFB and PEFC protocols, induced significant changes in the antioxidant profile of microgreens, with beetroot showing the most pronounced effects. When exposed to field strengths of 3 to 4 kV/cm, beetroot microgreens exhibited a 15% increase in TPC and a 12.4% enhancement in antioxidant capacity (ABTS). Ahmed *et al.* (2020) also reported significant increases in concentration of antioxidant compounds and TPC in wheat plantlet juice, after applying 2 to 6 kV/cm to the seeds. Curiously, no impact on DPPH inhibiting was found when PEF treatments of 0.5 to 2 kV/cm were applied to wheatgrass seeds as a pre-sowing technique (Leong *et al.*, 2016). These findings are consistent with previous studies indicating that PEF-induced membrane permeabilisation can trigger the generation of reactive oxygen species (ROS), a response to abiotic stress in plant cells (Leong *et al.*, 2016; Li & Burritt, 2003). The transient oxidative burst caused by ROS accumulation is believed to activate antioxidant defence pathways, often resulting in the biosynthesis of phenolic compounds and other secondary metabolites with protective functions (Sabri *et al.*, 1996).

2.2.2 TOTAL SOLUBLE SOLIDS

The results obtained for Total Soluble Solids (TSS), by direct measurement of °Brix of the prepared hydroalcoholic extract, are represented in Figure 24.

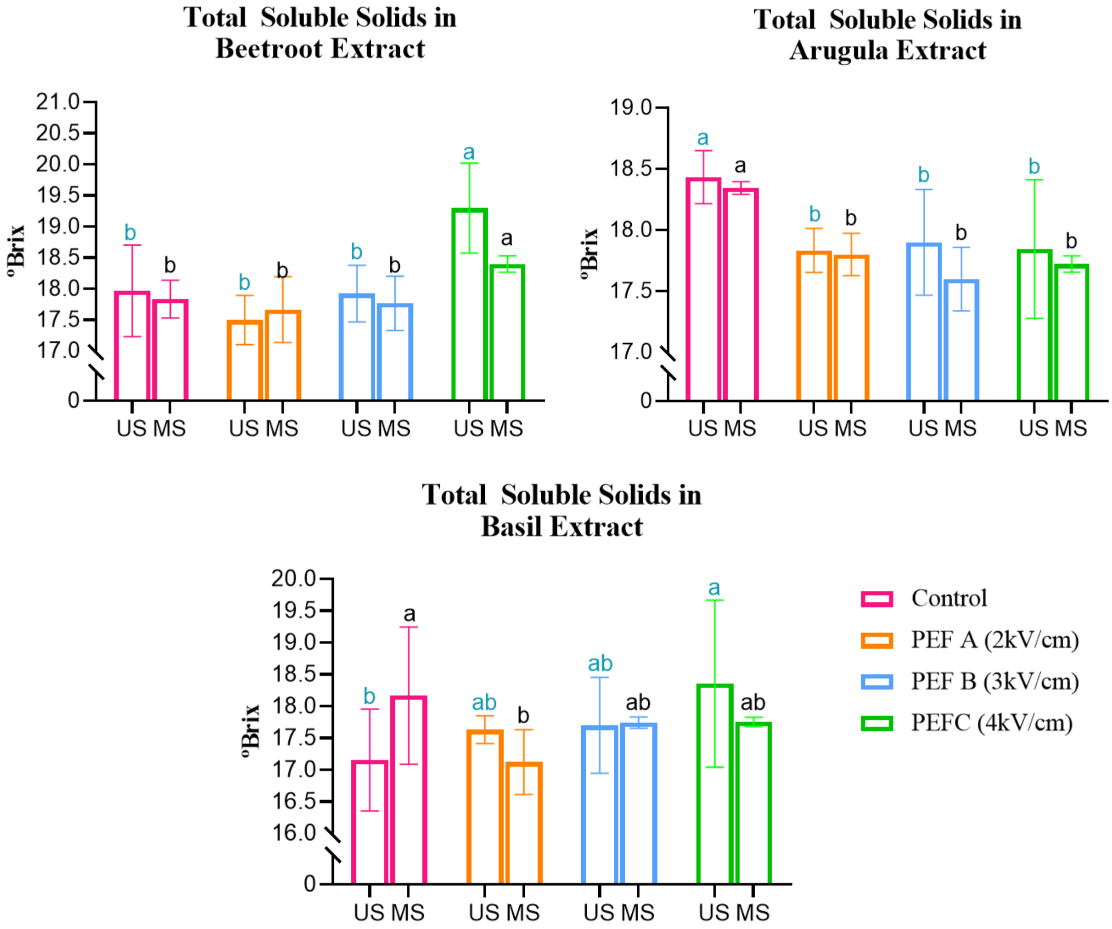


Figure 24. Total Soluble Solids in Beetroot, Arugula and Basil Microgreens. Statistically significant differences (Tukey's $p < 0.05$) are indicated by different letters assigned to the means. Statistical comparisons were conducted within each colour group. MS: Magnetic Stirring, US: Ultrasound.

The analysis of Beetroot extracts presented a consistent behaviour, with both MS and US extraction methods displaying significative higher °Brix values for extracts obtained from microgreens derived from seeds treated with the PEFC protocol (MS - Control: 17.8 °Brix vs PEF3: 18.4 °Brix; US - Control: 17.96 °Brix vs PEF3: 19.30 °Brix). This corresponds to an increase in TSS present in microgreens of approximately 3.4 and 7.5%, respectively.

The opposite was observed for Arugula, considering °Brix values were significantly higher in Control samples - both US and MS – compared to all PEF-treated groups, presenting a maximum decrease in TSS of -3.2% and -4%, for US and MS, respectively.

Basil presented intermediate results: in MS extracts, the highest TSS was found in the Control sample (18.43 °Brix), whereas in US extracts, the PEFC treatment yielded the highest value (18.34 °Brix).

Beetroot results are in line with a study conducted on *Scutellaria baicalensis* seeds, which showed that PEF treatment of 0.5 kV/cm significantly increased α-amylase activity, leading to the conversion of starch into soluble sugars during early growth (Song *et al.*, 2024). This suggests that PEF can accelerate cellular metabolism and promote sugar accumulation in seedlings. However, given the contrasting results observed in basil and arugula, the effect of PEF on sugar content appears to be species-specific and dependent on the applied treatment parameters. These findings support the theory of species-specific PEF requirements and the necessity for further in-depth studies on the mechanisms involved.

2.2.3 NEAR-INFRARED REFLECTANCE SPECTROSCOPY

After analysing the dried samples, the humidity parameter showed slight variations among them. Therefore, the samples were standardised to ensure a consistent basis for comparison. This also allows the results to be expressed either as a percentage or in g/100g of dried sample. After standardisation, the results were analysed, and the final data are presented in Table 17:

Table 17. Nutritional Parameters of the different PEF Protocols for Beetroot, Arugula and Basil**.**

	Parameter	Control	PEFA	PEFB	PEFC	*p*	η_p^2
Beetroot	*Fat*	2.92 ± 0.41	2.70 ± 0.30	2.06 ± 0.20	4.13 ± 1.58	0.084	0.545
	Protein	15.39^{b} ± 0.98	15.51^{b} ± 0.60	14.69^{ab} ± 0.29	20.72^{a} ± 3.90	0.023	0.678
	Crude Fiber	17.05^{a} ± 0.29	17.42^{a} ± 0.48	16.94^{ab} ± 0.35	12.84^{b} ± 3.13	0.024	0.672
	Ash	27.05^{a} ± 1.46	27.34^{a} ± 0.67	27.41^{a} ± 0.56	19.40^{b} ± 5.40	0.021	0.685
	Starch	27.81^{c} ± 0.74	27.50^{c} ± 0.64	32.54^{a} ± 0.58	30.63^{b} ± 0.47	<0.001	0.945
	Neutral Detergent Fiber	43.69^{a} ± 0.84	45.10^{a} ± 0.68	42.78^{ab} ± 0.44	35.87^{b} ± 5.87	0.022	0.680
	Lysine	2.32^{a} ± 0.14	2.35^{a} ± 0.09	2.30^{a} ± 0.03	2.01^{b} ± 0.06	0.005	0.778
	Cystine	0.35 ± 0.02	0.30 ± 0.01	0.29 ± 0.01	0.50 ± 0.22	0.155	0.462
	Methionine	0.20 ± 0.06	0.20 ± 0.03	0.11 ± 0.02	0.40 ± 0.22	0.083	0.546
	Phosphorus	2.03 ± 0.02	2.05 ± 0.04	2.02 ± 0.01	1.11 ± 0.87	0.074	0.560
Arugula	*Fat*	1.27^{c} ± 0.08	1.63^{b} ± 0.12	2.35^{a} ± 0.04	2.43^{a} ± 0.14	<0.001	0.971
	Protein	9.27^{bc} ± 0.17	9.03^{c} ± 0.11	10.32^{a} ± 0.32	9.56^{b} ± 0.08	<0.001	0.905
	Crude Fiber	6.87^{d} ± 0.13	7.19^{c} ± 0.07	8.97^{a} ± 0.08	8.49^{b} ± 0.07	<0.001	0.993
	Ash	7.80^{b} ± 0.65	7.98^{b} ± 0.07	8.57^{ab} ± 0.19	9.91^{a} ± 0.72	0.005	0.779
	Starch	32.00^{c} ± 0.33	34.37^{b} ± 0.49	35.85^{a} ± 0.42	35.49^{a} ± 0.29	<0.001	0.957
	Neutral Detergent Fiber	32.76^{a} ± 0.24	31.45^{b} ± 0.18	32.19^{a} ± 0.37	30.96^{b} ± 0.28	<0.001	0.905
	Lysine	1.58^{b} ± 0.03	1.59^{b} ± 0.02	1.45^{a} ± 0.02	1.47^{a} ± 0.02	<0.001	0.924
	Cystine	0.28^{a} ± 0.00	0.27^{a} ± 0.01	0.15^{c} ± 0.02	0.20^{b} ± 0.01	<0.001	0.974
	Methionine	0.11^{b} ± 0.01	0.15^{b} ± 0.01	0.13^{b} ± 0.01	0.19^{a} ± 0.02	0.001	0.854
	Phosphorus	0.59^{c} ± 0.01	0.66^{b} ± 0.01	0.74^{a} ± 0.01	0.76^{a} ± 0.02	<0.001	0.982

Table 17. Nutritional Parameters of the different PEF Protocols for Beetroot, Arugula and Basil**.**

	Parameter	**Control**	**PEFA**	**PEFB**	**PEFC**	***p***	η_p^2
Basil	*Fat*	4.44^{b} ± 0.02	4.44^{b} ± 0.06	5.07^{a} ± 0.13	5.00^{a} ± 0.21	<0.001	0.888
	Protein	22.87^{a} ± 0.12	22.13^{b} ± 0.23	22.26^{ab} ± 0.15	22.87^{a} ± 0.42	0.013	0.722
	Crude Fiber	11.77^{a} ± 0.19	11.21^{b} ± 0.08	11.40^{b} ± 0.13	11.10^{b} ± 0.10	0.001	0.850
	Ash	17.80^{a} ± 0.33	15.94^{b} ± 0.41	16.30^{b} ± 0.11	16.44^{b} ± 0.31	<0.001	0.887
	Starch	28.81^{b} ± 0.49	30.67^{a} ± 0.18	31.52^{a} ± 0.25	30.75^{a} ± 0.44	<0.001	0.918
	Neutral Detergent Fiber	34.18^{a} ± 0.47	33.08^{b} ± 0.16	32.65^{b} ± 0.04	32.52^{b} ± 0.14	<0.001	0.907
	Lysine	2.08^{a} ± 0.01	1.98^{b} ± 0.02	1.94^{c} ± 0.02	1.99^{bc} ± 0.03	<0.001	0.883
	Cystine	0.68^{a} ± 0.01	0.64^{b} ± 0.01	0.61^{c} ± 0.01	0.63^{bc} ± 0.01	<0.001	0.889
	Methionine	0.51^{bc} ± 0.00	0.49^{c} ± 0.01	0.54^{a} ± 0.01	0.53^{ab} ± 0.01	0.001	0.847
	Phosphorus	0.54^{b} ± 0.02	0.52^{b} ± 0.01	0.63^{a} ± 0.02	0.61^{a} ± 0.01	<0.001	0.906

NOTE: Statistically significant differences ($p < 0.05$) are indicated by different letters assigned to the means.
PEFA: 2 kV/cm, PEFB: 3 kV/cm, PEFC: 4kV/cm.

PEF significantly influenced the nutritional composition of Beetroot, Arugula, and Basil microgreens, with species-dependent effects on macronutrients and minerals. Given that the results are expressed in g/100g of dry weight, the observed changes reflect modifications in nutrient concentrations without interference due to moisture content, allowing the direct comparisons across treatments.

Beetroot suffered significant changes in several nutritional parameters under PEF treatments. *Protein* content increased significantly with PEFC application, presenting a 34.6% increase, of which 67.8% can be associated with the treatment. Additionally, *Crude Fibre* and *Ash* content decreased under PEF treatments, especially in the PEFC group, which had the lowest values. On the other hand, *Starch* content significantly increased, particularly with PEFB. Large effect sizes, η_p^2, were observed for *Starch* and *Lysine,* indicating substantial effects of PEF on these parameters.

For Arugula, PEF treatments led to significant increases in *Fat*, *Protein*, and *Starch* contents. Notably, *Protein* content increased 11.3% for PEFB microgreens, together with a *Crude fibre* increase of up to 30.6%. *Fat* content also increased significantly across all PEF treatments, with PEFB and PEFC showing the highest increases, from 1.27 g/100g (Control) to 2.35 g/100g and 2.43 g/100g, respectively. Interestingly, *Lysine* levels decreased slightly under the PEF treatments, with PEFA and PEFB showing a reduction to 1.45 g/100g and 1.47 g/100g, respectively, compared to the Control value of 1.58 g/100g. The most significant changes were in *Fat* (η_p^2= 0.971) and *Starch* (η_p^2= 0.957), indicating the strong effect of PEF on these parameters.

For Basil, *Protein* content showed a slight decrease of -3.2% when seeds were subjected to PEFA protocols; In opposition, PEFB and PEFC protocols remained statistically similar to the Control. While *Fat* content was unaffected by PEFA protocol, it increased significantly, up to 14.2% under PEFB and PEFC, with a large effect size of η_p^2= 0.888. *Crude Fibre* content decreased under all PEF treatments, with PEFC presenting the lowest value, 5.7% lower than Control. *Starch* content increased slightly under all PEF treatments, peaking with PEFB protocol, where a 9.5% increase was observed compared with Control seeds.

Fiber Content results are particularly interesting, considering that *Crude Fiber* generally decreased in comparison with Control: -24.7% in Beetroot and -5.7% in Basil (PEFC), and 30.6% in Arugula (PEFB). The concentration of *Neutral Detergent Fiber* (NDF) was also diminished, with the most relevant differences being -17.9% in Beetroot, -5.5% in Aru-gula, and -4.8% in Basil, all observed when seeds were subjected to 4 kV/cm. Given *Crude Fiber* mainly consists of cellulose and lignin, a reduction in its concentration can contribute to digestibility improvement. Cellulose and lignin are concentrated in cell walls, and their reduction can increase microgreen nutrient density and decrease satiety sensation, thereby also increasing intake rates. In contrast, *NDF* also includes hemicellulose, which humans have more difficulty degrading; ruminants, however, can utilize through degradation and fermentation. (Weimer, 2022). NDF decreased in all species, treated with PEFC (Beetroot: -17.9%, Arugula: -5.5%, Basil: -4.8%). In addition, the *CF/NDF* ratio in Beetroot decreased from 39% to 36% when subjected to a 4 kV/cm protocol, which might be interpreted as an improvement in digestibility, since a higher relative proportion of hemicellulose implies that vegetable cell walls present a more soluble composition. While this is less relevant regarding human consumption, it would be interesting to extrapolate to other agronomic and study the possible positive impacts ruminant feed. Arugula and Basil presented opposite trends, where *CF/NDF* increased from 33.5% to 34% in Arugula, and from 21% to 27% in Basil, with PEFC protocol.

Regarding amino acids, *Lysine* decreased in PEF-treated samples for all species by up to 13%, while *Methionine* increased in all species: up to 100% in Beetroot, 72% in Arugula, and 5.8% in Basil. *Cystine* concentration was species-dependent, increasing by 42% in Beetroot treated with 4 kV/cm, but decreasing in Arugula (–46%) and Basil (–10%).

With the exception of Beetroot, where *Phosphorus* content decreased under PEFC treatment (–45%), its concentration increased by up to 28% in Arugula and 16.6% in Basil.

Across all species, PEFB and PEFC treatments generally had the most significant impacts on nutritional parameters.

In summary, PEF treatments led to significant changes in the nutritional content of all three species, with Beetroot showing a notable increase in *Protein* and a decrease in *Crude fibre* and *Ash* content, particularly under the PEFC treatment. Arugula exhibited increases in *Fat, Protein*, and *Starch,* while Basil showed an increase in *Fat* content and slight decreases in *Crude Fibre*. Across all species, PEFB and PEFC treatments generally had the most significant impacts on nutritional parameters. These results indicate that PEF has the potential to effectively influence the nutritional profiles of microgreens, with species-specific variations in response to different treatment protocols.

2.3 POST-HARVEST: SENSORY ANALYSIS

The results of the comparative sensory analysis performed on the three species under study - beetroot, arugula, and basil -, to assess possible organoleptic differences are represented in radar charts, one for each of the species. Observing the obtained sensory profiles for each species, together with the data present in Appendix I and its statistical analysis, it is possible to draw some conclusions.

The results of the organoleptic analysis for Beetroot microgreens are discriminated in Figure 25.

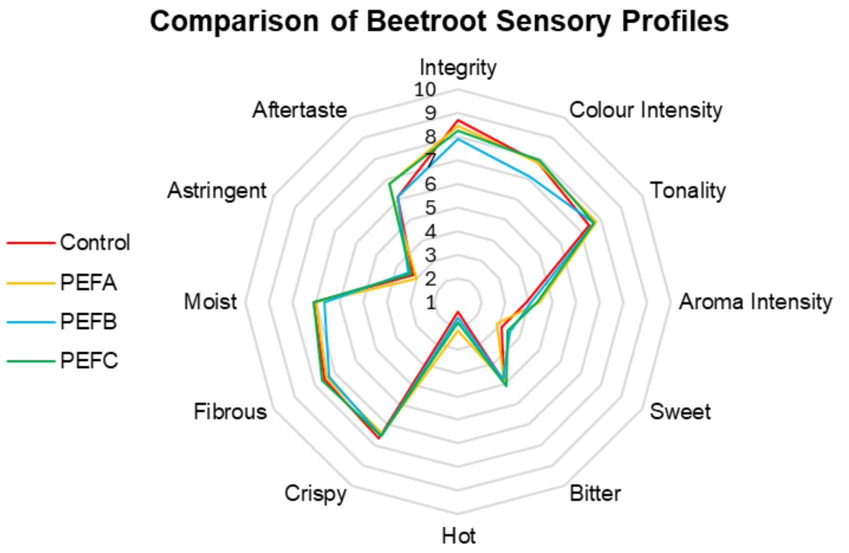


Figure 25. Comparison of Beetroot Sensory Profiles.

For instance, Beetroot's sensory profile was not heavily affected by any of the PEF treatments, with the most pronounced differences being reported for *Colour intensity*, with PEFB subjects demonstrating a slight reduction compared to other treatments. *Sweet* is possibly one of the most appealing and considered attributes when discussing beetroot; PEF treatments above 3 kV/cm seem to enhance this attribute, as PEFC and PEFB received the highest scores. In contrast, *Tonality* remained relatively stable across all groups. *Aroma Intensity* was enhanced in all beetroot microgreens treated with PEF, being this difference being more prominent in PEFA and PEFC. *Crispy*, *Hot*, *Fibrous*, *Moisture,* and *Astringency* attributes showed minor variations but remained consistent across treatments. In addition, the lingering effect regarding *Aftertaste* seemed to persist for longer in microgreens obtained from PEFA and PEFC-treated seeds. These observations are corroborated by the conducted statistical analysis, considering that no significant differences were found between treatments for this species (Pillai's Trace = 0.677, $F_{(36, 69)}$ = 0.559, p = 0.971, η_p^2 = 0.226).

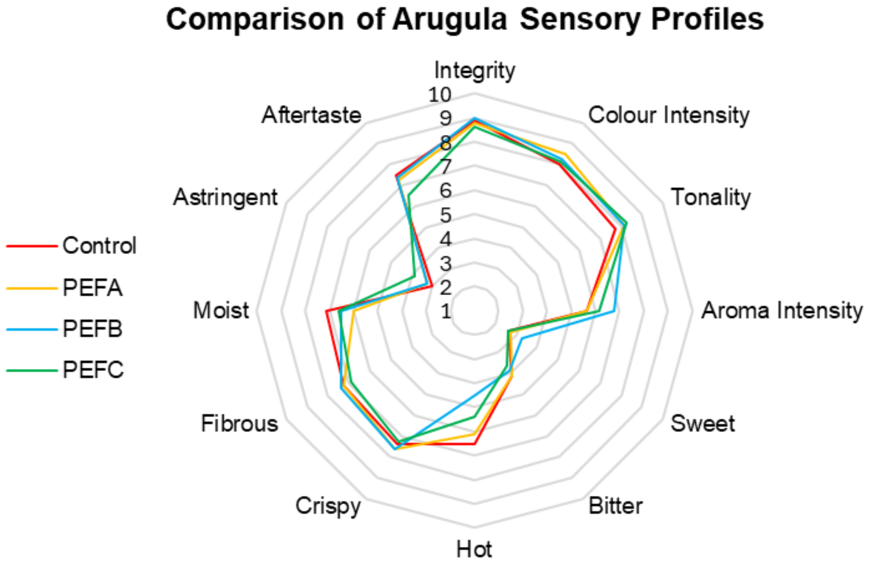


Figure 26. Comparison of Arugula Sensory Profiles.

Regarding Arugula's comparative sensory analysis (Figure 26), panellists identified the most noticeable differences in *Aroma Intensity*, with PEFB receiving the highest score, followed by PEFC, while subjects of the Control and PEFA groups exhibited lower similar values. One of the primary interests in terms of curiosity was determining if the *electropriming* had any effect on the commonly characteristic arugula's *Hot* attribute. In fact, a reduction of the pontuation for this attribute was found for subjects, as Control got the highest score. Similarly, *Aftertaste* presented the same behaviour. In opposition, *Astringency* was slightly more noticeable in PEFC. Like Beetroot, *Colour Intensity* and *Tonality* presented small fluctuations but remained generally consistent across treatments. Statistical analysis demonstrates that, while these differences are noticed, they are not statistically significant; however, the medium effect size of the treatment was the highest amongst the three species, being able to explain 41.5% of the assessed differences between treatments (Pillai's Trace = 1.245, $F_{(36, 57)}$ = 1.124, p = 0.341, η_p^2 = 0.415).

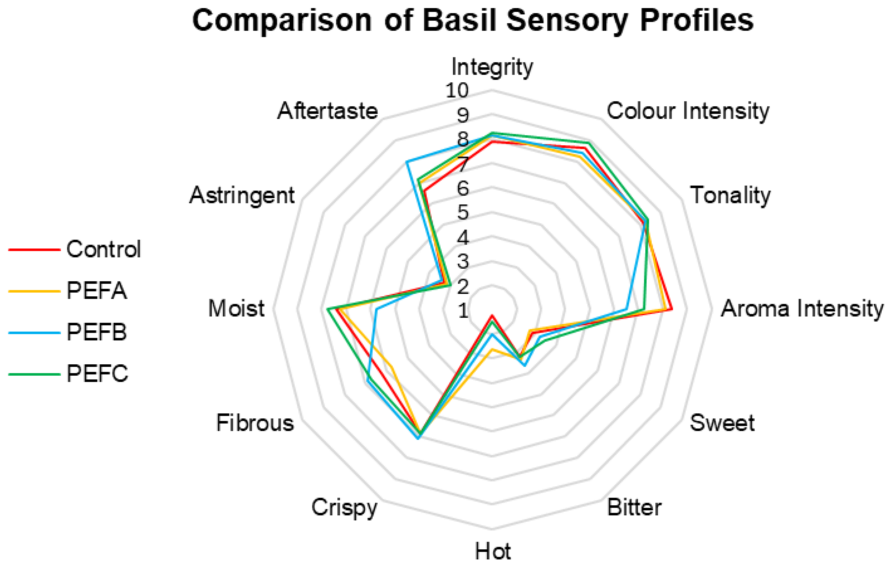


Figure 27. Comparison of Basil Sensory Profiles.

The comparative sensory analysis of Basil also revealed interesting results (Figure 27): similarly to the other species, descriptors *Colour Intensity* and *Tonality* remained consistent across treatments. Different treatments appeared to affect *Aroma Intensity*, considering that higher scores were attributed to Control, while PEFB presented the least fragrant sample. In contrast, a more intense PEF protocol seems to increase the perception of *Sweet*, considering its successive increase of scores up to PEFC. *Astringency*, *Bitter*, and *Crispy* attributes seem not to be affected by the pre-sowing treatments. Equally to the other counterparts, statistical analysis indicated that the observed differences are not statistically different, with 32.4% of the detected variation explained by the different PEF treatments (Pillai's Trace = 0.973, $F_{(36, 57)}$ = 0.760, p = 0.809, η_p^2 = 0.324).

Overall, while some findings suggest that some sensory attributes can be influenced by the different PEF protocols applied as a pre-sowing treatment, statistical analysis reveals that these differences are not significant. However, these results suggest that that minor effects can take place on specific sensory attributes, as in *Sweet* for Beetroot, *Hot* for Arugula and *Aroma Intensity* for Basil, which indicates a potential need for further research on this subject. Ideally, future studies should employ tasting panels specifically trained for these descriptors, food category and species, which could let to more robust and significant conclusions.

III. FINAL CONCLUSIONS AND FUTURE PERSPECTIVES.

This chapter was mainly focused on the realisation of a comprehensive study with a multidisciplinary approach consisting of the application of Pulsed Electric Fields to seeds of three different species – Beetroot, Arugula, and Basil. The experimental design consisted of a comparative study on the production of Microgreens, resorting to Control subjects and three different PEF Protocols: PEFA (2 kV/cm), PEFB (3 kV/cm) and PEFC (4 kV/cm).

The evaluation of water uptake and electrical conductivity during the imbibition of Basil, Beetroot, and Arugula seeds under different PEF treatments revealed several trends and species-specific responses. Seed imbibition, evaluated by Water Uptake of the seeds and Electrical Conductivity of the water, used to both kickstart seed metabolism and for PEF Application, was significantly influenced by the PEF priming protocol used. PEF-treatment led to significantly higher water uptake by Beetroot, when seeds are subjected to 3 and 4 kV/cm (PEFB and C) in comparison with Control, presenting an effect size of the treatment of 45.6% for seed weight. Beetroot σ was higher for all treated subjects. The same was observed for Arugula, considering that PEFA and PEFC exhibited an increase of conductivity 21% superior to Control (η_p^2=0.727), immediately after PEF-treatment. However, the differences diminished over time, being only observed for PEFA at the 4-hour mark. Basil was mostly affected by 3 and 4 kV/cm protocols, given that PEFB and PEFC presented significantly higher σ in comparison to Control and PEFA, with a η_p^2=0.926 at the 4-hour mark. The results indicated that PEF treatments modulated water absorption, with Beetroot and Arugula exhibiting significantly higher conductivity values and enhanced imbibition under specific protocols. These findings suggest that electroporation induced by PEF may have facilitated water uptake, potentially accelerating metabolic activation.

Regarding Germination indexes and Radicle Length, a Linear Mixed Model analysis revealed that treatment consistently and significantly affects radicle length across all species (Beetroot: p=0.004; Arugula: p<0.001; Basil: p<0.001. Differences in both radicle length and the number of germinated seeds were evident from day 1 and remained consistent over the period of 5 days. This suggests that PEF seed priming may have a lasting effect on early plant development, which could have implications for crop establishment and yield potential. According to the results, the ideal PEF protocols are PEFC for Beetroot, PEFB for Arugula, and PEFB/C for Basil.

PEF treatments significantly reduced both Chlorophylls and carotenoids in Beetroot and Arugula, with Arugula showing a large effect size (η_p^2=0.731), while Beetroot's effect size was smaller (30.6%). In contrast, Basil showed an increase in pigment concentrations with more intense PEF protocols (PEFB and PEFC), although the treatment effect was lower (42.6%). Overall, PEF reduced pigment concentrations in most species, but for Arugula, the PEFB protocol showed a notable increase in radicle length and germination, suggesting enhanced tissue development may contribute to the pigment changes, as it might be associated with a greater dispersion. In addition, for basil, these results may indicate that progressively higher electrical field strengths lead to an increase in pigment biosynthesis.

Results from Total Phenolics, Total Soluble Solids, Antioxidant Capacity by DPPH and ABTS Scavenging assays and Total Soluble Solids performed in 70/30 hydroalcoholic extracts obtained with the aid of 2 different preparation methodologies, US and MS. For Beetroot, extracts obtained with the MS technique presented no differences between subjects. However, when considering US extracts, PEFB protocol presented the maximum increase in the concentration of TPC, +15% in comparison with Control. (p=0.020). A similar concentration increase was found for radical scavenging activity, considering DPPH antioxidant capacity increased 10.8 (US) to 12.4% (MS) when a protocol of 3 or 4 kV/cm was applied (PEFB & PEFC). For ABTS, all PEF-treated subjects presented significantly higher antioxidant capacity than Controls. For Arugula, DPPH assays also reported a +5% increase over control when seeds were subjected to PEFC treatment. For ABTS assay, no significant differences were found in US extracts, while MS presented the highest concentration of antioxidants for PEFC. While Basil DPPH results showed high antioxidant activity across all samples, PEFC significantly enhanced antioxidant activity in MS extracts by 2.6% (p<0.001; η_p^2 = 0.527). Although the relationship between PEF-induced ROS and enhanced antioxidant metabolism in plants is not yet fully understood, this data supports the theory that mild PEF exposure may be a viable strategy to promote the accumulation of health-promoting compounds in some species.

Regarding TSS, Beetroot extracts showed higher °Brix values with PEFC treatment in both MS and US methods (+3.4 and +7.5%). In contrast, Arugula had higher °Brix in Control samples (less 3.2 to 4% with PEF). Basil showed mixed results.

Lastly, it was possible to conclude that extraction efficiency differed significantly between MS and US aiding methods. In a generic way, higher extraction rates were found when extracts were prepared with ultrasound (p<0.001). However, much like the remaining results, this effect presents species-dependency. Considering the example of Basil, DPPH and ABTS scavenging assays presented distinct conclusions, which leaves us with a possible conclusion: the impact on ABTS-reactive compounds is not as dependent on PEF seed *electropriming* as DPPH.

Regarding the nutritional constitution of Microgreens, PEF treatments significantly affected the nutritional composition of Beetroot, Arugula, and Basil microgreens, with species-specific responses. Beetroot showed an increase of +34% for *Protein* and +41% *Fat* content, when subjected to PEFC (4 kV/cm), while *Starch* improved +17% with PEFB protocol. Similarly, Arugula experienced increases in *Fat* (+91%, PEFC), *Protein* (+11%, PEFB), and *Starch* (+12%, PEFB). Basil saw increased *Fat* content (+14%) with PEFC, while *Crude Fibre* augmented up to 9.4% in PEFB. PEF influenced Fiber content in a potentially interesting form in Beetroot microgreens: with the most intense treatment, PEFC (4 kV/cm), *Crude Fiber* reduced -24.7%, while *Neutral Detergent Fiber* reduced 17.9%. Furthermore, the results indicate a reduction in CF/NDF ration, from 39 to 36% in Beetroot microgreens treated with PEFC. Considering the reduction of these concentrations, it's possible to conclude that PEF may prompt the development of cell walls with a higher soluble composition (i.e. hemicellulose), which can promote consumption and enhance digestibility rates. Results for Arugula displayed the same trends, with Crude Fiber diminishing up to -30.6% (PEFB) and Neutral Detergent Fiber up to -5.5%; however, CF/NDF increased from 33.5 to 34%. For Basil, PEFC saw a reduction in Crude Fiber content of -5.7% and -4.8% in Neutral Detergent Fiber; CF/NDF augmented from 21% to 27.4%. With respect to amino acid contents, lysine decreased in PEF-treated samples for all species by up to 13%, while methionine increased in all species: up to 100% in Beetroot, 72% in Arugula, and 5.8% in Basil. Cystine concentration was species-dependent, increasing by 42% in Beetroot treated with 4 kV/cm, but decreasing in Arugula (–46%) and Basil (–10%). With the exception of Beetroot, where phosphorus content decreased under PEFC treatment (–45%), its concentration increased by up to 28% in Arugula and 16.6% in Basil. Overall, PEFB and PEFC had the most significant effects on nutritional parameters across species, suggesting that PEF can enhance microgreen nutritional profiles, potentially enhancing their nutritional value.

PEF treatments had minor but noticeable effects on the sensory profiles of Beetroot, Arugula, and Basil, though statistical analysis revealed no significant differences. *Sweet* was enhanced in Beetroot with PEF treatments above 3 kV/cm (PEFB, PEFC), while *Aroma Intensity* was highest in the Control and lowest in Basil (PEFB), and increased in Arugula (PEFB, PEFC). The characteristic *Hot* attribute of Arugula was slightly reduced in PEF-treated samples, and *Colour*

Intensity and *Tonality* remained stable across treatments for all species. For the *Sweet* descriptor of Beetroot, it is noteworthy that these results are consistent with the TSS analysis, which showed a significant °Brix increase in PEFC extracts. These findings suggest that, while PEF may subtly influence specific sensory attributes, it does not adversely affect the overall sensory quality of microgreens. Further studies would be valuable, ideally employing a specialized microgreen tasting panel trained on specific species and descriptors.

Overall, this study provides a comprehensive analysis of the effects of PEF seed priming on microgreens, integrating physiological, biochemical, nutritional, and sensory assessments. The findings highlight species-specific responses, with PEF treatments influencing seed imbibition, germination dynamics, pigment composition, and nutritional profiles, while sensory attributes remained largely stable, suggesting that further studies are needed to understand the underlying mechanisms and refine treatment parameters for specific crops. Optimal protocols appear to be PEFC for Beetroot, PEFB for Arugula, and PEFA/B for Basil. PEF seed priming emerges as a valuable tool for enhancing microgreen quality.

Future studies should focus on elucidating the mechanisms of PEF-induced modifications at the cellular and molecular levels. Additionally, research should be expanded to assess the scalability of PEF applications in commercial microgreen production, considering economic feasibility and energy efficiency. Further investigations into the long-term effects of PEF on plant metabolism and post-harvest quality would also be beneficial. By refining PEF parameters and exploring their interactions with different plant species, this technology has the potential to become an integral part of sustainable agricultural practices, contributing to improved crop performance and nutritional value in the food industry. Given the promising results obtained in this study, it is plausible to consider the potential extrapolation of PEF seed priming effects to other crop species and production systems. While this work focused on microgreens, partly because this model allows a “fast-track” model, considering the small turnover time, the observed enhancements in water uptake, germination, and nutritional composition suggest that similar benefits could be achieved in larger-scale vegetable or cereal crops. Future research should explore the adaptability of PEF technology to diverse agronomic systems, including its potential to enhance seedling vigour, crop resilience, and overall yield under varying environmental conditions

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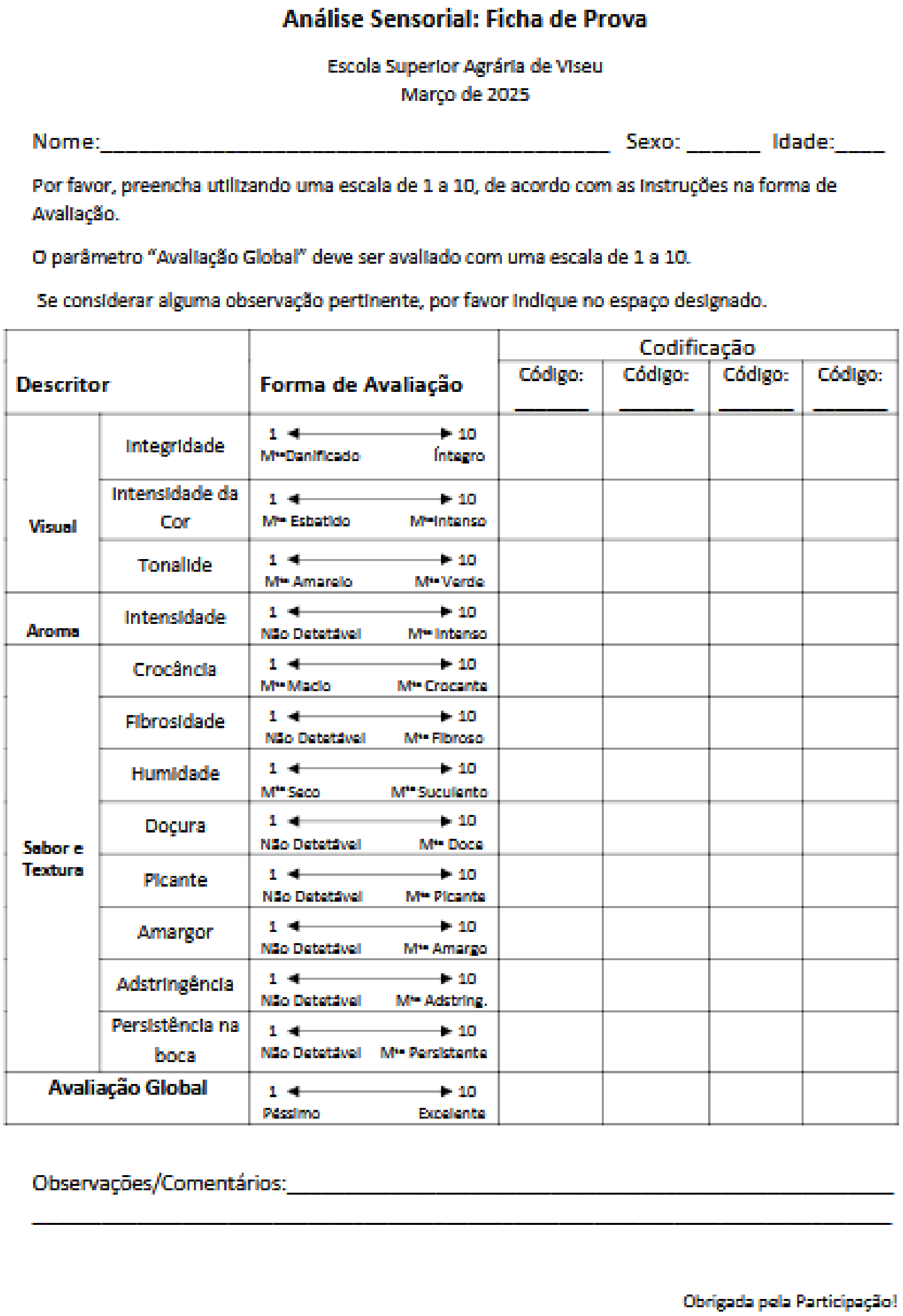
APPENDIX

Appendix I



Análise Sensorial: Ficha de Prova

Escola Superior Agrária de Viseu
Março de 2025

Nome:____ Sexo: ______ Idade:____

Por favor, preencha utilizando uma escala de 1 a 10, de acordo com as instruções na forma de Avaliação.

O parâmetro "Avaliação Global" deve ser avaliado com uma escala de 1 a 10.

Se considerar alguma observação pertinente, por favor indique no espaço designado.

Descritor		**Forma de Avaliação**	Codificação			
			Código: ______	Código: ______	Código: ______	Código: ______
Visual	Integridade	1 ◄——► 10 Mto Danificado Íntegro				
	Intensidade da Cor	1 ◄——► 10 Mto Esbatido Mto Intenso				
	Tonalide	1 ◄——► 10 Mto Amarelo Mto Verde				
Aroma	Intensidade	1 ◄——► 10 Não Detetável Mto Intenso				
Sabor e Textura	Crocância	1 ◄——► 10 Mto Macio Mto Crocante				
	Fibrosidade	1 ◄——► 10 Não Detetável Mto Fibroso				
	Humidade	1 ◄——► 10 Mto Seco Mto Suculento				
	Doçura	1 ◄——► 10 Não Detetável Mto Doce				
	Picante	1 ◄——► 10 Não Detetável Mto Picante				
	Amargor	1 ◄——► 10 Não Detetável Mto Amargo				
	Adstringência	1 ◄——► 10 Não Detetável Mto Adstring.				
	Persistência na boca	1 ◄——► 10 Não Detetável Mto Persistente				
Avaliação Global		1 ◄——► 10 Péssimo Excelente				

Observações/Comentários:____
____

Obrigada pela Participação!