UNIVERSITAT POLITÈCNICA DE VALÈNCIA

School of Agricultural Engineering and Environment

ELECTROTHERMAL AND ACOUSTIC TECHNIQUES APPLICATION IN EXTRACTION PROCESSES. STATE OF THE ART

End of Degree Project

Bachelor's Degree in Food Science and Technology

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Title: Application of electrothermal and acoustic techniques during the process of extraction. State of the art.

Abstract:

A great amount of the food industry wastes includes important content of bioactive compounds, which can be extracted and applied in different sectors such as food, chemical or pharmaceutical areas. The application of alternative technologies can improve the extraction of interesting compounds, enhancing the process, reducing the energy consumption, or limiting the use of organic solvents.

The objective of the present study was to stablish the state of the art of the application of different electrothermal and acoustic techniques to intensify the extraction processes. The eco-friendlier techniques on which this study was focused were Moderate Electric Fields (MEF)/Ohmic Heating (OH), Pulse Electric Fields (PEF) and Ultrasound (US). The methodology applied was based on the analysis of different studies carried out by researchers, comparison, and exemplification, to reach conclusions in relation to the objective of the study. The use of the electric field-based techniques during the extraction process produces a phenomenon in cell membranes known as electroporation. This phenomenon means the permeabilization of the cell membrane, which can be related with a reduction of extraction time, the increase of yield, as well as the possibility of use of more environmentally friendly solvents. Ultrasound application produce different effects such as cavitation, which can intensify the solvent penetration into the solid matrixes, enhancing mass transfer processes and then the release of the bioactive compound.

Therefore, the techniques studied can contribute to the increase of the extraction of bioactive compounds. In this sense, the influence of process variables and product characteristics must be considered. Therefore, research must be done to better understand the mechanism involved for each specific product/application and optimize the operation.

Keywords: extraction, Moderate Electric Field (MEF), Pulse Electric Field (PEF), Ultrasound, electroporation, cavitation.

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Título: Aplicación de técnicas electrotérmicas y acústicas procesos de extracción. Estado del arte. / Aplicació de tècniques electrotèrmiques i acústiques en processos d´extracció. Estadi de l'art.

Resumen:

Una gran cantidad de los residuos de la industria alimentaria incluyen gran cantidad de compuestos bioactivos, que pueden ser extraídos y aplicados en diferentes sectores como el alimentario, químico o farmacéutico. La aplicación de tecnologías alternativas puede mejorar las operaciones de extracción, intensificando el proceso, reduciendo el consumo de energía o limitando el uso de solventes orgánicos.

El objetivo del presente estudio fue establecer el estado del arte de la aplicación de diferentes técnicas electrotérmicas y acústicas para intensificar procesos de extracción. Las técnicas en las que se centró este estudio fueron los campos eléctricos moderados (MEF)/calentamiento óhmico (OH), los campos eléctricos pulsados (PEF) y los ultrasonidos (US). Las técnicas basadas en aplicación de campos eléctricos durante el proceso de extracción producen un fenómeno en las membranas celulares conocido como electroporación. Este fenómeno puede inducir la permeabilización de la membrana celular, lo que puede implicar una reducción de los tiempos de extracción, un aumento del rendimiento, así como posibilitar el uso de disolventes orgánicos más respetuosos con el medioambiente. La aplicación de ultrasonidos puede producir la aparición de cavitación, entre otros efectos. Esto puede contribuir a una mejora en la penetración del solvente en las células, una mejora en la transferencia de materia y por lo tanto una intensificación de la extracción de componentes bioactivos de interés. Por lo tanto, las metodologías consideradas pueden contribuir al incremento de la extracción de compuestos bioactivos. En este sentido, se deben considerar todas las variables de proceso, tanto las correspondientes al proceso mismo como las características propias de la matriz en cuestión. Por ello, se necesita realizar estudios concretos para cada aplicación/producto específico para comprender los mecanismos implicados y optimizar las operaciones.

Palabras clave: extracción, campos eléctricos moderados (MEF), campos eléctricos pulsados (PEF), ultrasonidos, electroporación, cavitación.

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First, I would like to thank my tutor, Juan Andrés Cárcel Carrión, for having helped me throughout this process, for his trust and for giving me the opportunity to develop my work with total freedom.

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INDEX

INDEX OF FIGURES

INDEX OF TABLES

1. INTRODUCTION

According to the Food Agricultural Organization (FAO) 38% of the world's land area is dedicated to agriculture, which means approximately 5.000 mega-hectares. Globally, the distribution of agricultural land depends on the combination of climatic, edaphic, and economic factors. Thus, the largest proportion of agricultural land is concentrated in Asia, hosting 34%, followed by America (25%) and Africa (24%), while Europe and Oceania both accounted 20%.

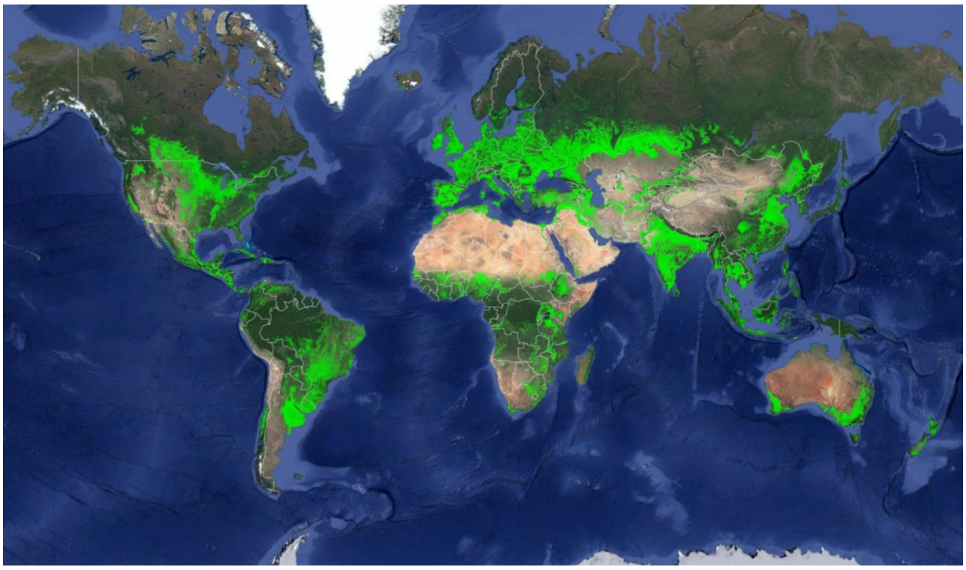

Figure 1. Cropland distribution across the world according to the United States Geological Survey (USGS, 2017).

Because of this huge activity, an enormous amount of waste is generated during harvesting, post-harvesting, processing, and consumption of food products. So, one of the main problems in agri-food industries is how to manage all the waste generated throughout the supply chain, from the beginning of the growth of the crop, until it uses by the consumer. Due to the linear economy model followed until now, most of the waste generated has the disposal as a unique destination. However, the change to a circular economy model involves the maintenance of the products in the production/consumption/recycle chain at much time as possible to reduce or eliminate the amount of waste produced by the system (Barros et al., 2020).

Around the world, the transition into a circular economy is not developed and uniform as would be desirable and depends on a variety of different factors such as the degree of industrialization, the level of technological development, the availability of qualified human resources and financial access, among others. However, in recent years the circular economy has been growing, as showed by the adoption of some measurements in Europe (McCourtie, S. 2021). This can be considered an action to promote the compliance of the Sustainable Development Goal (SDG) number 12 ensuring sustainable consumption and production patterns, specifically with the target 12.2, which, wants to achieve the sustainable management and efficient use of natural resources by 2030.

In this sense, a great percentage of the food industry wastes include important content of bioactive compounds, which can be extracted and used in different sectors such as food, chemical or pharmaceutical areas. The application of alternative technologies can improve the extraction operations, for example, reducing energy consumption or the use of organic solvents. This is in line with the SDG 9, which ensures to build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation, contributing to a decrease of the environmental impact of the activity. Thus, the current study aims to compare different electric and acoustic techniques to the intensification of the extraction process of interesting, significant, and useful biocomponents.

1.2 Principle of extraction

The solid-liquid extraction operation is used to separate components from a solid matrix using a liquid solvent (de Oliveira et al., 2015). The extraction procedure can be divided into several steps: immersion of the sample, where the interesting compound is found, in the extraction solvent; impregnation of solvent into solid matrix; dissolution of the compound into the solvent; and extracted compound transport, jointly the extraction solvent, through the solid matrix until the solid surface and then to the surroundings (Mustafa & Turner, 2011). The last step is the separation of the compound from the solvent.

The mass transfer process depends on several factors, among which it can be highlighted the resistance that substances found during the migration through the cell membrane (Puértolas et al., 2012) and the resistance to the movement of the solvent and solvent-extract complex inside the solid matrices which is mainly attributed to diffusion mechanisms induced by a concentration gradient.

The extraction process can take long time, being this fact mainly affected by the process variables that affect the mass transfer such as temperature, particle size, affinity between target compounds and solvent, accessibility of the solvent to the solid matrix, etc. In this sense, electric and acoustic techniques described in this work may help in the performance of this process, improving product quality and shortening extraction time.

1.2.1 Main constraints of extraction operation

Extraction is one of the most common unit operations in food industry (Both et al., 2014), since it is a simple procedure which allows to obtain interesting compounds from different matrixes (Arenas, 2018). For extraction processes, it is important to determine the most adequate solvent to extract the component of interest. In this sense, most of the conventional extraction processes are based on the use of organic solvents, such as methanol, chloroform, hexane, isopropanol, acetone, or diethyl ether. These solvents are very effective but can produce a high environmental impact. Moreover, their management involves important expenses in security measures and recovering operations. For these reasons, there is a great interest to find alternatives, which permit to avoid their use.

Furthermore, extraction is usually a low efficiency process, requiring a great amount of energy. In addition, the semipermeable structure of cell membrane limits the diffusion of the compounds, thus increasing the time required for the extraction. All these facts made that the food industry is under pressure to develop more efficient extraction processes, which apply moderate conditions and maximize the product quality.

1.3 Application of Electric Fields in the Food Industry

The use of electrical treatments in food processing has shown a sustained progression during the last few decades (Basset & Vorobiev, 2001). There are different techniques based in the use of electric energy, but the current study was focused in only two of them, the application of pulsed electric fields (PEF) and moderate electric fields (MEF).

1.3.1 Pulsed Electric Field (PEF)

Pulsed electric field (PEF) is a non-thermal food processing technique that involves the application of short electric field pulses of high intensity to a food matrix (Pollini et al., 2021). The duration of the electric field pulses can vary from milli to microseconds and the electric field intensity applied ranges from 0.1 kV·cm^{-1} to 100 kV·cm^{-1} (Martín, 2018). The PEF treatments are carried out inside a chamber provided with two electrodes, separated a distance one from each other, the shorter the distance the stronger, the electric field applied. These electrodes must be made up of a material that does not interact with the food (e. g. different titanium alloys). One of them is connected to a high-voltage generator and the other is a ground connection. When the generator is on, a potential difference is produced between the two electrodes, giving rise to the formation of a high intense electric field. The application of this electric field is made in pulse way. The main function of the chamber is to ensure the uniformity of the electric field throughout it (Martín, 2018).

The application of these high intensity electric pulses induces instability and tension in the cell membrane of the material subjected to the treatment (Vivanco et al., 2021). This fact produces changes in the structure of the cell membrane, favoring pore formation, which increases the membrane permeability (Thamkaew & Gómez, 2020) and makes easier the material diffusion through the membrane via electro-osmosis (Knirsch et al., 2010). This phenomenon is called as electroporation, and it can be reversible or irreversible depending on the cell properties, such as size or shape, and the process parameters considered (Thamkaew & Gómez, 2020), such as electric field strength, temperature, and process time (Gavahian et al., 2018). Reversible electroporation is produced when cell is able to regenerate the membrane and recover its viability after the treatment (Martín, 2018, Thamkaew & Gómez, 2020). This process can be used to introduce/extract some components to/from the inner of a cell or induce cell stress, which start metabolic paths for the production/destruction of other compounds.

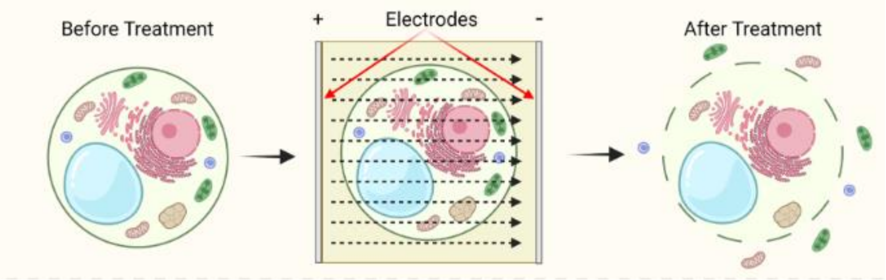

Figure 2. Irreversible poration in cell membrane produced by the application of PEF (Naliyadhara et al., 2022).

However, when the intensity of the electric field increases, the pores formed have greater stability due to their larger size (Figure 2), causing the cell is unable to repair the damage (Martín, 2018). Thus, the treatment may cause lethal damages to the cell due to irreversible changes of the cell membrane permeability and the leakage of cytoplasmic contents.

1.3.2 Moderate electric field (MEF)

Although there is not a fixed formal definition of moderate electric field, it can be stated that this technique involves applying an alternating electric current without pulsation (Gavahian et al., 2018), using arbitrary waveforms (Sensoy & Sastry, 2004) at moderate electric intensity. Thus, in this case, the electric field strength could be ranged from 1 to 1000 $V \cdot cm^{-1}$. In these conditions, it can also be induced electroporation in cells. The application of an appropriate voltage through the matrix produces the rupture of the membrane in the form of pores and therefore increases its permeability (Jaeschke et al., 2016).

Another important effect of MEF application is the heating effect due to the Ohm law. Thus, when an alternating electric current passes through a material, this acts as an electric resistance, increasing its temperature due to the Joule effect. The heating occurs in the form of internal energy transformation, from electric to thermal (Knirsch et al., 2010). In fact, some applications of this technique are called as ohmic heating (OH). The heating is uniform in products with uniform composition, on the opposite of the non-uniform temperature distribution that appears in convection or microwave heating (Varghese et al., 2014) being this, one of the main advantages of OH. Therefore, the MEF treatments can be focused in the electroporation effect, in the thermal effect or in both (Lozano, 2021). In fact, MEF application has been applied in extraction processes because it can promote electroporation of the cell membranes, increasing its permeability, but also is an efficient way to achieve the desired temperature for the extraction operation.

1.3.3 Specific parameters of pulsed electric field technique

Some of the process variables have similar impact in both PEF and MEF applications. This is the case of electric conductivity of treated products, electric field strength applied or electric energy input. These parameters will be described in the following section. However, there are other specific factors of PEF treatments, such as specific energy input, pulse number or pulse width (Puértolas et al., 2012) which are describe here.

1.3.3.1 Electric energy input

The electric energy input is expressed in $kJ \cdot kg^{-1}$ and represent the ratio between the electric energy applied and the amount of product treated. In PEF application is usually lower than the typical values needed for MEF processes (Lebovka et al., 2007). In the case of PEF, it depends on the applied voltage, shape, width and number of the pulses, volume of the treatment zone and resistance of the treatment chamber (Martín, 2018).

1.3.3.2 Pulse characteristics

Pulse shape

The type of pulse applied depends on the capacitors, resistors, and type of switches of the system, being square and exponential decay the most common pulse shapes used in PEF application (Rivas, 2012). The square wave pulse (Figure 3) is characterized by a fast increase of the potential at the beginning of the process until reaching the working voltage, the maintenance of this potential over time, and rapidly decreasing it until reach the 0 value. The exponential decay type of pulse (Figure 4) reaches the maximum potential quickly followed by a gradual decrease until achieve the potential value of 0. This gradual decay represents a partial drawback for PEF applications because part of the electrical energy has no effects on electroporation and only contributes to the heating of the food, which usually is not the aim this application. In contrast, in square wave pulses, the energy used is applied to the maximum working voltage (Rivas, 2012).

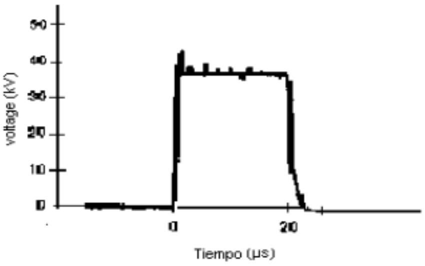

Figure 3. Square wave pulse (Rivas, 2012)

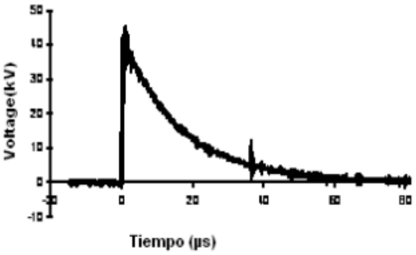

Figure 4. Exponential decay pulse (Rivas, 2012)

Pulse width

The width of the pulse varies depending on the type of pulse shape considered. In the square wave pulse, the width of the pulse corresponds to the duration of the pulse, which is the duration of the peak amplitude (Naliyadhara et al., 2022). However, in the exponential decay pulse, the width is defined by the time during which the voltage is greater than 37% of the maximum value of the potential reached, because the voltage varies with the duration of the pulse (Rivas, 2012). This parameter is expressed from nanoseconds to milliseconds.

Number of Pulses

As specified in the name of PEF, the electric field is applied in a pulsed way. Therefore, the number of pulses (N) is one of the key parameters to be considered (Naliyadhara et al., 2022) because is directly related with in the intensity of the treatment. Thus, the higher number of pulses applied, the greater energy applied and the more intense the PEF effect, which can affect the extraction capacity.

Pulse frequency

In the case of PEF application, the frequency is the number of pulses per unit of time, and it is expressed in Hz. It is an important parameter to be considered since it determines the amount of electric energy applied on the treated product per unit of time (Martín-García et al., 2020).

1.3.4 Process variables in moderate electric field applications

Among the parameters that can influence the effect of MEF application in process, such as extraction it can be highlighted temperature, electric frequency, electric field strength, electric conductivity, process time and raw material characteristics (Gavahian et al., 2018).

1.3.3.1. Electric conductivity

Electric conductivity is a property of the material being treated. It is one of the most important parameters to consider, because it is directly linked to the MEF and PEF effects. It can be described as the ability of a matrix to conduct electric energy and depends on food structure, chemistry, and temperature. The electric conductivity can be affected by ionic strength, free water, and material microstructure. Thus, it is greatly influenced by the presence of ionic substances such as salts or acids (Varghese et al., 2014).

Moreover, the application of an electric field can induce diffusion through the cell membrane, which can modify the conductivity of the medium, and then the effects of MEF. The increase of temperature also induces an increase of conductivity. In his sense, the preheating of some foods makes them more suitable for MEF technique application (Varghese et al., 2014).

1.3.3.2 Electric field strength

Electric field strength is a quantitative expression of the intensity the electric field applied. It is defined as the ratio of the potential between two electrodes and their distance (equation 1):

$$\mathrm{E} = \frac{\mathrm{V}}{\mathrm{d}} \qquad \textit{(Eq.1)}$$

Where E is the electric field strength (V/cm); V, the potential (V); and d, the distance between the two electrodes (cm) (Rivas, 2012).

1.3.3.3 Electric energy input

The electric energy input determines the magnitude of the MEF effects during the process (Buckow et al., 2011). It is expressed in $kJ \cdot kg^{-1}$ and is defined as the electrical energy applied per unit of mass (Naliyadhara et al., 2022). It noticeably increases with the increase of the electric field strength, and it is mainly related to the rise of temperature produced by the effect of ohmic heating (Lebovka et al., 2007).

1.3.3.4 Frequency

Unlike PEF, in MEF application, which uses alternating current (AC) (Lozano, 2021), frequency (f) is defined as the number of times the alternating current changes its polarity. The unit of measurement is hertz (Hz, number of cycles per second). The frequencies typically used in MEF application are in between 50 – 60 Hz. Higher frequencies can be applied to limit degradation reactions in electrodes, but the lower ones favor the effect of electroporation (Varghese et al., 2014).

1.3.3.5 Temperature

Temperature can influence on the cell damage. High temperatures can induce changes in cell membrane fluidity and in the structure of the cell walls. In this sense Puértolas et al. (2012) from microscopic analysis reported some cell wall disruption, increasing cell's sensibility and favoring the solubility of the extracted compounds. Furthermore, viscosity and surface tension are influenced by thermal energy, decreasing when temperature increases. Additionally, high temperatures improve the interaction between the solvent and the matrix, improving the extraction process. So, the increase of the temperature contributes to the enhancement of the mass transfer into the solvent (Machado et al., 2019).

During MEF process, temperature must be controlled, since thermolabile substances can be degraded or denatured when temperature rises. Therefore, in some cases, e. g. extraction operations, it could be necessary to consider refrigeration systems, such as cool jackets, to keep the desired value of temperature throughout the process (Torgbo et al., 2022).

1.3.3.6 Raw material characteristics

Within the raw material characteristics that can influence the effects of MEF application it can be mentioned the particle size, heat capacity, and viscosity. Thus, in particles of 15-25 mm, the orientation relative to the electrical field has a significant influence on the electrical properties. The size of the particles can also influence the heat capacity, since they form different phases, and the particle size influences the heating rate of those phases. Furthermore, in solid particles when all of them have similar electrical conductivities, the component having lower heat capacity will heat faster (Varghese et al., 2014).

Regarding viscosity, foodstuffs with high viscosity show faster ohmic heating than those that present low viscosity (Varghese et al., 2014). This could be linked to the temperature dependence of electrical conductivity. Thus, fluids with higher viscosity do not easily transfer their energy to phases with lower conductivity, such as particulate phases (Khalaf. W et al., 1996).

1.4 High intensity ultrasound

The application of high intensity ultrasound is known to have significant impact on the rate of mass transfer, which is involved in many food processes (Chemat et al., 2011). Thus, one of the most interesting applications of this technique is the extraction of bioactive compounds from agro-food matrices (Ahmad-Qasem et al., 2013). Ultrasonic waves induce mechanical vibrations when traveling across a biological material, producing successive expansion and compression cycles. These cycles can induce the bubbles formation in liquid media. This phenomenon is called cavitation and can be stable or inertial. Thus, once formed the bubbles, there is gas incursion inside the bubble during expansion phase. After, during the compression phase, part of this gas is expelled. However, there is a net gas gain in the microbubble. For this reason, the microbubbles continue increasing their size until they reach the resonant size and produce a stable oscillation of the microbubbles. Such stable oscillations create a microstreaming (Lentacker et al., 2014). Inertial cavitation occurs at higher ultrasound intensities. Due to the higher oscillation amplitude, the microbubbles increase their size until reach a critical value. Then, they implode and fragment into many smaller microbubbles producing a very high temperatures and pressures in very local points and during very little time. If these collapsing microbubbles are close to solid surface, such as a cell membrane, an asymmetrical collapse takes place and the shock waves generate extremely high forces capable of perforating the solid matrix (Lentacker et al., 2014). These mechanical and thermic effects can cause particle reduction size, damage of the cell walls and microstructure modification.

Moreover, in solid matrixes, the compression and expansion of solid materials induce the so-called "sponge effect" and the formation of internal micro channels. All of this contributes to the intensification of the solvent penetration into the cells (Figure 5), the release of the bioactive compounds from the cell and the enhancing of mass transfer (Jovanović et al., 2017).

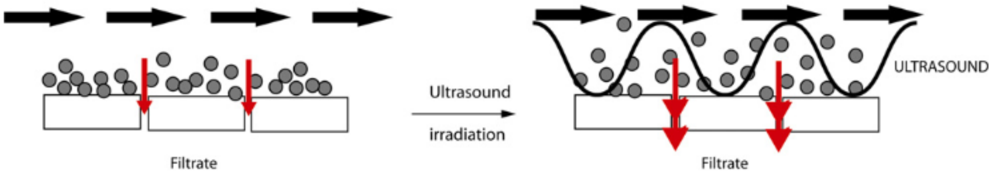

Figure 5. Enhancement of permeability using ultrasound (Chemat et al., 2011).

1.4.1 Main parameters in high intensity ultrasound applications

The main variables to be considered in the use of ultrasound in processing such as extraction processes are the applied ultrasonic power and the frequency, but other process variables, such as temperature, can also affect the magnitude of effects.

1.4.1.1 Ultrasonic power

The ultrasonic power is the amount of acoustic energy applied to the medium and is expressed in watts (W). Generally, it is expressed as the electric power supplied to the ultrasound transductor, because it is easier to measure than the real acoustic power introduced to the medium. In the industry, it can be found equipment that can module the electric power applied between 0 and 100% of the power of the equipment.

Usually, the greater the power applied to the transducers the stronger vibration generated, causing a more intense acoustic field. Therefore, the effects of ultrasound increased, thus intensifying the cavitation and enhancing mass transfer.

1.4.1.2 Frequency

The ultrasonic frequency is defined as the number of times than a particle is in a particular position per unit of time, and it is expressed in Hz. Then, it measures the number of alternating compression and expansion cycles per unit of time. The applications of high intensity ultrasound take place usually at low frequency range, 20-100 kHZ. This is due to the increase of ultrasonic wave attenuation at greater frequencies, which limits the intensity of ultrasonic effects. Moreover, at lower frequencies, larger cavitation bubbles are produced and are greater the effects induced (Esclapez et al., 2011).

1.4.1.3 Temperature

The application of ultrasound in liquid media produces a temperature rise due to the friction produced by the effect of the wave's expansion and compression cycles. This increase of the temperature leads to higher dilution of the interesting compounds, so they can be released. However, it can affect the quality of the extracted compounds, such as producing the denaturation of the proteins if the temperature rises until critical values (Chemat et al., 2011).

In addition, in extraction operations, it is important consider other process variables such as the type of solvent, the solvent-sample ratio, or the particle size (Esclapez et al., 2011)

2. MATERIALS AND METHODS

To carry this bibliographic work, the main articles related with the topic was consulted in the following databases:

- Web of Science (Elsevier)
- Scopus
- Google scholar
- Polibuscador

The method used was to check in these databases the following keywords and their combination to refine searching: Pulsed electric fields (PEF), Moderate electric fields (MEF), Ultrasound, extraction, extraction yield, solvent, temperature, time, voltage, frequency, electric field strength, energy input, pulses, ultrasound, power and environment.

Once all the articles of interest were obtained, they were classified in folders, by the type of technology addressed, MEF, PEF o Ultrasound, making easy them to order the specific information extracted for each technique. Subsequently, a further classification was carried out, identifying the methodologies, the process variables used, the statement of the results obtained and conclusions. Thus, it was possible to order the information and extract the influence of the different parameters of each technique in extraction operations.

3. MEF APPLICATION IN EXTRACTION PROCESSES

Moderate Electric Field (MEF) technology and its relationship with technological processes in food is being increasingly studied. The use of this technique is more commonly focused to thermal applications, such as cooking, thawing, drying and even pasteurization since it is considered as a more effectiveness alternative to the conventional indirect heating methods. Regarding the influence of MEF application in extraction operations, it is important to analyze the influence of conditions tested. In this sense, the different process variables considered in the literature when applying this technique are the following.

3.1 Voltage

Voltage is one of the main variables to be considered in extraction processes assisted by MEF application. Thus, there is a direct relationship between the increase of voltage gradient and the increase of the yield and/or extraction kinetics. This is because at greater voltage gradient, the potential difference produced in the cell membrane causes the formation of pores, that is, the phenomenon of electroporation. This pore formation facilitates the mass transfer and enhances transmembrane diffusion, thus increasing extraction yield. De Oliveira et al. (2015) observed this fact on pectin extraction from samples of passion fruit peel. In this study, a MEF pre-treatment was applied at different voltage (30, 50 and 100 V), for a fix time of 15 minutes and with an extraction temperature controlled at 45 ± 2ºC. It was observed that the pectin extraction yield when applying MEF at 30 V (4.98 g/100g of peel d.m,) was 20 % lower than at 100V (6.20 g/100g of peel d.m.). Kulshrestha et. al (2002) also reported an enhancement of diffusion induced by the increase of the voltage applied. Thus, they used three different levels (5, 10 and 20 V) in a betanin extraction process from beetroot. While the application of 5 V was ineffective, the application of higher voltages instead significantly improved the mass transfer of betanin through the solvent. Thus, at 20 V it was found an increase in extraction of 95% when compared to 5 V experiments. The authors attributed this fact to the electroporation induced by MEF.

In contrast, Jaeschke et al. (2016) found no significant influence in MEF application in lipid extraction from *Heterochlorella luteoviridis* (ethanol as solvent) when applying different voltage gradients. The voltage used for this study varied from 0 to 180 V. The results concluded that irreversible electroporation did not occur, likely due to the low electric conductivity of ethanol, which can cause the electrical energy delivered to the medium to be insufficient to induce electroporation of the cell membrane.

3.2 Electric field strength

As explained above, voltage in MEF and in PEF application can affect both the yield and extraction kinetics. However, not only it is important the electric potential difference applied but also the distance between electrodes, being both variables included in the electric field strength variable. Thus, Boonchoo et al. (2014) and Coelho et al. (2019) agree to conclude that the higher electric field strength the greater damage produced in the cell walls of the materials treated. Boonchoo et al. (2014) studied the effect of the MEF application in the extraction of lime oil at different electric field strengths (25, 50 and 100 $V\cdot cm^{-1}$) and a fixed temperature of 60 ºC. They reported that an increase in the electric field strength applied from 25 to 100 $V\cdot cm^{-1}$ reduced the time of extraction from 4 min to approximately 14 s. Coelho et al. (2019) tested three different electric field strength, 4, 6 and 11 $V\cdot cm^{-1}$, in the extraction of bioactive compounds (carotenoids) from tomato. With the application of 11 $V\cdot cm^{-1}$ they observed an increase of 30% of total carotenoid yield (14µg/g of biomass) when compared to experiments carried out at 4 $V\cdot cm^{-1}$ (10µg/g of biomass). However, the application higher electric field strength could lead to a degradation of the sensitive compounds. Pataro & Ferrari (2021) conducted a study about the extraction of juice and their by-products from blueberry fruits by using MEF. They studied the influence of temperature (from 40 to 80 ºC) and electric field strength (0, 18, 36 and 54 $V\cdot cm^{-1}$). Thus, the yield obtained for 18 $V\cdot cm^{-1}$ experiments was 1683 mg of total anthocyanins/L of juice, being this value increased to 2277 mg/L when the applied electric field strength reached to 36 $V\cdot cm^{-1}$, therefore an increase of 26% in extraction was obtained. All these results pointed to the fact that at the higher electric field strength applied, the more effective the extraction. However, Pataro & Ferrari (2021) also reported that the application of high electric fields strength led to a reduction in the extraction yield of total anthocyanin content from blueberry fruits juice. This effect was also observed by Pereira et al. (2016) (Table 1) in their study about the influence of MEF application in the extraction of bio-active compounds from colored potato. This can be attributed to the fact that the application of MEF improved yield extraction reducing the extraction time. However, the application of an excessively intense MEF treatment could cause the partial destruction of the compound to be extracted. Therefore, regarding the effectiveness of the application in an extraction process, there is an optimum electric field strength for each specific case.

3.3 Frequency

Frequency of alternant current used is another electric variable that can affect the influence of MEF application in extraction processes. Thus, Lima and Sastry (1999) found that frequency increased the juice extraction yields from apple samples. The samples were submitted to a MEF treatment at a frequency 60 Hz and the extraction yield was compared with that obtained from raw non-treated samples. MEF treatment at 60 Hz resulted in higher juice yield (586.9 ml/kg) than the untreated sample (486.4 ml/kg). This means an increase of 17.1 % between untreated samples and MEF treated samples at 60Hz. Therefore, these authors concluded that the effect of frequency applied during MEF treatment was significant. Lakkakula et al. (2004) studied the extraction of rice bran oil using the MEF technology. This study was carried out by using 10 g of rice bran, an electric field strength of 100 V·cm^{-1} and two frequencies, 1 Hz and 60 Hz. The results of the study showed that the extraction yield increased from 50 to 80% when the frequency was dropped from 60 to 1 Hz. The improvement in the extraction yield was not caused by an increase in the temperature, but due to the electroporation induced by the electric treatment.

Recently, Gavahian et al. (2022) studied the effect of MEF application in pineapple core valorization. Different times (15, 30, 45 and 60 min), temperatures (70, 80, 90 and 100ºC), voltages (110, 160, 210 and 260 V), and frequencies (60, 340, 620 and 900 Hz) were used in the study. The results obtained showed that the higher frequency applied the lower the energy consumption and the greater the antioxidant extraction. However, the MEF experiments carried out at lower frequency showed greater losses of Total Polyphenol Content (TPC). These contradictory results indicates that, the influence of MEF frequency is different depending on the product and each case have to be studied individually.

3.4 Solvent

Solvent used is a key factor in an extraction operation. Obviously, the solvent chosen has to be able to solubilize the solute to be extracted, but other transport properties such as viscosity or diffusivity must be taken into account. In the case of MEF assisted extraction, the solvent electric conductivity is essential to determine the intensification of extraction and the solubility of the bioactive compounds (Jaeschke et al., 2016). Coelho et al. (2019), studying the MEF assisted extraction of bioactive compounds from tomato by-products, found that the extraction yield increased using the ethanol/water mixture as solvent compared to water. These authors observed a significant increase in the extraction of total polyphenols up to 2.821 ± 0.211 mg gallic acid equivalents/ g when using a mixture of ethanol/water of 70% v/v (70°C, 15 min) compared to the control experiment, the MEF assisted extraction with water. The authors attributed these results to the fact that these by-products are rich in lipophilic compounds that can form complexes with polyphenols. This can improve their solubility in ethanol, which present lower polarity than water, and enhance the diffusion process through the unipolar cell walls.

This fact was further supported by Jaeschke et al. (2016). These authors performed experiments about lipid and carotenoid extraction from *Heterochlorella luteoviridos* applying MEF and using ethanol as solvent. They maintained the voltage gradient applied at 180 V, varied the concentration of the ethanol/water solution (25/100, 50/100 and 75/100 v/v) and measured the carotenoid extraction yields. Their results showed a carotenoid extraction yield using as solvent an ethanol/water solution of 25/100 and 50/100 v/v of 2% and 7% respectively, while the extraction yield using ethanol/water at 75% v/v reached to 73%. As for lipid extraction, it was observed the same trend, the lower the proportion of ethanol in the solvent, the lower the extraction of lipids. In this case, the extraction yield ranged from 3% using ethanol/water at 25/100 v/v to 8% at 75/100 v/v. The influence of the proportion of ethanol in solvent was also observed in MEF treatments at lower voltage. Thus, at 90 V the carotenoid and lipid concentration extracted with a 75/100 v/v ethanol/water solution was higher than those obtained with a 50/100 v/v one, which means that the greater the ethanol concentration, the higher the lipids extraction yield. However, it is important to highlight that the application of MEF was the main factor to improve the yield (Jaeschke et al., 2016). Therefore, the presence of water in the solvent, even at the lower proportion tested, is enough to conduct the electric current and induce the effects of MEF.

Sometimes the solvent used includes ions, which can affect electric conductivity and then, MEF effects. For some experiments, there are specific extraction methods and MEF can be used as a pre-treatment of extraction. Thus, Nair et al. (2014) conducted a study of extraction of oil from rice bran using MEF as a pre-treatment in a solution of sodium chloride. Afterwards they carried out the solvent extraction with n-hexane, as it is the most viable solvent for the extraction. They analyze the influence of MEF application and the concentration of salt in the pretreatment solution (from 0.01 M to 1 M NaCl solution). The results showed that the application of the MEF pre-treatment reduced the extraction time by 70%. In addition, when analyzing the extraction performance with n-hexane, the lower the concentration of sodium chloride in the MEF pretreatment solution, the shorter the extraction solvent time needed and the better the quality of the extracted oil. The electrical conductivity is one of the main parameters in MEF application. Thus, the introduction of ionic salts, such as sodium chloride in solvent, contribute to modify its electrolyte concentration and then its electric conductivity increasing the MEF effects in the process.

3.5 Treatment time

Treatment time must be considered a key parameter in extraction operations, specifically in those assisted with MEF because its relationship with the use of facilities and energy consumption. As a rule, the longer the treatment the greater the extraction capacity. However, an uncontrolled treatment time can cause problems, such as excessive cell damage and deterioration of the product quality, for example by increasing the lipid oxidation (Gavahian et al., 2018).

Thus, Pare et al. (2014) studied the oil recovery from soybean seeds. The experimental plan included the study of multiple variables, among which was the treatment time. The parameters considered for the study were applied voltage (600, 750 and 900 V), temperature (70, 80 and 90 ºC) and time (0, 5 and 10 min). The results indicated that the maximum oil recovery (73 %) was obtained when enzymatic hydrolyzed soybean slurry was held at 600 V and 90 ºC for 10 min of treatment, shorter processes meant lower yield. This fact was also observed by Oliveira et al. (2015) studying the extraction of pectin from passion fruit peel at different treatment time (5, 15, 40 and 60 min). They found the content of galacturonic acid extracted was greater when extraction time increased from 5 to 15 min (47.77 and 68.72 g/100g d.m, respectively). However, the yield obtained after 40 min of process was not significantly different from the obtained at 15 min, and even decreased after 60 min of treatment. This fact could be due to an excess in the treatment time, which can degrade temperature-sensitive compounds and worsen the quality of the pectin.

Coelho et al., (2019) carried out experiments using different application times (0, 15 and 30 minutes), ethanol/water solvent at different proportions (0, 35 and 70 v/v) and temperatures (40, 55 and 70ºC) during the extraction of lycopene from tomato by-products using MEF (4, 6 and 11 $V\cdot cm^{-1}$). They conclude that the best extraction time, was 15 min (55ºC and 35% of ethanol). According to these authors, the longer time application considered (30 min) resulted in a greater degradation of lycopene. Therefore, the results obtained for different authors seem to indicate the existence of an optimum treatment time. Bellow it, the extraction is uncompleted and above it the quality of the extract can deteriorate.

In summary, the different studies published indicates that MEF could be a viable alternative to conventional extraction processes. As a general rule, it can be concluded that the increase of the MEF voltage and the strength of the applied electric field and the lowering of the frequency results in an increase of the extraction yield, and a decrease of the energy consumption. Moreover, the extraction could be improved with an adequate solvent and the application of the optimum treatment time. In any case, there are some exceptions and, therefore, to apply this type of process, all the variables must be carefully analyzed for each specific product.

Table 1. MEF treatment application in extraction processes.

Author	Sample	Extracted components	Operating conditions	Comments
Boonchoo et al., 2014	Lime	Lime Oil	E = 25, 50, 70 and 100 V·cm^{-1} T = 60ºC V = 150-950 f = 50 Hz	- Ohmic heating at E = 50 V·cm^{-1}could enhance yield of extraction. - Increasing electrical field strength decreased ohmic treatment time and increased the degree of tissue damage.
Coelho et al., 2019	Tomato peels and seeds	Polyphenols	E = 4, 6 and 11 V·cm^{-1} t = 15 min Solvent = ethanol/water at 70%	- Rate of recovery 58% higher than control samples. - Better extract quality and quantity.
de Oliveira et al., 2015	Passion fruit peel	Pectin	V = 30, 50 and 100 V f = 60 Hz t = 5, 15, 40 and 60 min pH extractant= 0.3, 1, 2 and 5 ± 0.02 Extractant ratio= 1:30 (v/w) T < 50ºC	- The maximum yield extraction was obtained at 100 V - The ideal extraction time was 15 min. - Higher extraction was observed at lower pH and electric voltage (50V).
Gavahian et al., 2022	Pineapple core	Polyphenols and antioxidants	T = 70, 80, 90 and 100 ºC t = 15, 30, 45 and 60 min V = 110, 160, 210 and 260 V f = 60, 340, 620 and 900 Hz	- Temperature is the most critical parameter which affects the energy consumption of the experiment. Temperatures over 80 ºC deteriorated the phenolic compounds. - Total phenolic content degradation occurred when extraction time increased at temperatures above 80 ºC. - There is a positive correlation between voltage and TPC (Total polyphenolic content) - Higher frequencies increased the extraction yield of TPC

Jaeschke et al., 2016	*Heterochorella luteoviridis*	Carotenoids and lipids	V = 0 – 180 V f= 60 Hz Ethanol/water solution= 25-/100 v/v T < 35ºC	- Maximum carotenoid yield (73 %) at 180 V of MEF combined with a 75/100v/v ethanol/water solution. - MEF showed no effect on lipid extraction, while ethanol/water solution (75/100 v/v) showed that up to 83% of the total lipid content can be extracted.
Kulshrestha et al., 2002	Beets	Betanin	t = 3 min T = 45ºC V = 5, 10 and 20 V	- Extraction was enhanced with higher electric field strength. - At 20 V it was found an increase in extraction of 95% when compared to 5 V experiments.
Lakkakula et al., 2004	Rice	Rice bran oil	E = 60, 100 and 140 $V \cdot cm^{-1}$ f = 1 and 60 Hz	- Increase of the electric field strength from 60 $V \cdot cm^{-1}$ to 100 $V \cdot cm^{-1}$ enhanced the extraction yield. - The higher extraction yield was obtained at the lower frequency tested. - Higher lipid extraction yield was obtained using MEF when compared to non-treated samples.
Lima et al., 1999	Red apples	Apple juice	E = 10, 60 and 70 $V \cdot cm^{-1}$ f = 60 Hz	- Higher extraction yield was obtained using 60 Hz when compared to non-treated samples.

Nair et al., 2014	Rice bran	Rice bran oil	Sodium chloride solutions= 0.01 M, 0.1 M and 1M V = 180 V C = 5, 10 and 20 A t = 1, 2 and 3 min	- Extraction time reduced by about 70% - Maximum oil extraction at 1M, 20 A and 3 min.
Pare et al.,	Enzymatic hydrolyzed soybean slurry	Soy oil	E = 600, 750 and 900 V T = 70, 80 and 90 ºC) t = 0, 5 and 10 min	- The highest extraction was obtained using 600 V and 90 ºC for 10 min of treatment. - The extraction recovery was also higher when MEF parameters where used.
Pataro et al., 2021	Blueberry fruit	Polyphenols and anthocyanins	E = 18-55 $V\cdot cm^{-1}$ T = 25 – 80 ºC	- Higher electric field strength conducted to higher extraction yield. - Increase in the extraction yield when increasing the temperature. - Anthocyanin is time and temperature dependent, important to establish a suitable value for these parameters.
Pereira et al., 2016	Colored potato	Anthocyanins	E = 0, 15 and 30 $V\cdot cm^{-1}$ T = 30, 60 and 90 ºC T= 0, 5 and 10 min	- Anthocyanins extraction increases with treatment time and electric field, but we must ensure a suitable value of electric field to avoid regressions.

E = electric field strength, f = frequency, T = temperature, t = time, V = voltage

4. PEF APPLICATION IN EXTRACTION PROCESSES

Like MEF, Pulsed Electric Fields (PEF) technology is being increasingly used in the food industry. PEF can be applied as a pretreatment of an extraction process. If irreversible electroporation occurs, this can favor the mass transport and make easier the extraction of compounds of interest. However, it must be considered the influence of process variables to apply this technique more effectively.

As stated in the case of the application of MEF technique in extraction operations, the solvent highly affects the extraction yield of the principal bioactive compounds. However, the PEF treatment could help the solvent action and permit the use of alternative solvents with environmental lower impact. Moreover, the electric characteristics of the applied field it has also to be considered.

4.1 Electric field strength

The electric field strength is one of the key parameters to be considered in PEF applications. In this sense, several studies have shown that the increase of this parameter intensifies the PEF effects likely linked to a greater induction of the cell membrane rupture (Naliyadhara et al., 2022). Thus, Andreou et al. (2020) pretreated olive pomace with PEF at two levels of field strength (0.3 and 6.5 $kV \cdot cm^{-1}$) before the extraction of polyphenols and proteins at room temperature using a 50% ethanol/water solution as solvent. PEF-treated samples increased 92% the yield of extracted polyphenols and proteins. These authors reported that above 3 kV/cm the phenomenon of electroporation is produced, intensifying the extractability. Luengo et al. (2013) studied the enhancing of the extraction of polyphenols of orange peel by the pulsed electric field application. These authors found that the higher electric field strength applied, the greater the cell disintegration index observed. This variable is a measurement of the level of electroporation induced by the treatment and considerably improved the extraction yield.

Segovia et al. (2015), also reported the influence of the electric field strength. They studied the aqueous extraction of polyphenols from borage (Borago officinalis L.) leaves pretreated with PEF at different electric field strengths (1, 3, 5 and 7 $kV \cdot cm^{-1}$). It was observed that an increase in the electric fields strength from 1 to 5 $kV \cdot cm^{-1}$ resulted in the increase of the cell disintegration index. Further increments of electric field strength above 5 $kV \cdot cm^{-1}$ did not increase this index. Therefore, it is shown that a specific electric field strength is needed to carry out specific treatments with PEF application.

To sum up, the increase of the electric filed strength applied enhances the electroporation and therefore improves extraction processes. However, an excess of the electric field applied can also cause the release of some undesired compounds during the extraction (Aouir et al. 2015). This means that it is necessary to determine for each application the optimum electric field strength to achieve the more effective extraction.

4.2 Energy input

The level of electroporation could be also affected by the effective energy input (U). At the same number and type of pulses, there is a relationship between the electric field strength and the specific energy input. Thus, it could be expected that an increase of the electric field strength increases the specific energy input, and therefore induces an increase in the extraction yield. Thus, Eing et al. (2013) studied the influence of this parameter in lipids extraction from microalgae

Auxenochlorella protothecoides. The PEF treatments (35 kV/cm, pulse duration of 1 µs) were carried out in a range of specific energy input from 50 kJ·kg^{-1} to 200 kJ·kg^{-1}, and a range of frequencies from 1 Hz to 4 Hz. The results revealed that the amount of intracellular stored biomass, TOC (organic carbon content) and carbohydrates, as well as the concentration of these in the extracellular medium, significantly increased with a PEF application of only 50 kJ·kg^{-1} compared to the sample without PEF pre-treatment. The increase of the specific energy applied until 150 kJ·kg^{-1}, increased the yield of extraction obtained. Above this specific energy, no additional yield was found.

Bobinaitė et al. (2015) studied the application of PEF in the production of juice and the extraction of bioactive compounds from blueberry fruits and their by-products. They tested the application of total specific energy input of 1, 5 and 10 kJ·kg^{-1} at a constant frequency of 10 Hz and a pulse duration of 20 µs. Their results also concluded that the specific energy input correlated with extraction yield. This fact can be explained because, in the range of the PEF treatment studied, there is a correlation between the permeabilization degree and the specific energy input.

The influence of the energy input was also observed by Andreou et al. (2020). They studied the extraction of high-added-value compounds from olive pomace, comparing the total polyphenol content of extracts obtained from non-treated and treated with different energy input samples. The final concentration for polyphenols and proteins, both intracellular compounds, did not exhibit significant differences between PEF-treated samples bellow a 4.85 kJ·kg^{-1} energy input and untreated samples. Above this value, the extraction yield was enhanced by PEF depending on the energy input applied. Thus, for PEF energy inputs from 7.27 kJ·kg^{-1} to 34.10 kJ·kg^{-1} the final phenolic and protein concentration extraction increased up to 20% and 33% when compared to untreated samples respectively. The highest phenolic concentration was found at 24.24 kJ·kg^{-1} energy input, being 42% higher than untreated sample.

4.3 Frequency

In PEF applications, the frequency factor refers to the number of pulses applied per unit of time (Puértolas et al., 2012). The electrical conductivity of biological cells has been found to be frequency dependent, low frequencies increasing the conductivity of the cell tissue because of electroporation (Puértolas et al., 2012). This conclusion was also reported by Segovia et al. (2015) studying the aqueous extraction from borage (*Borago officinalis* L.) leaves. Thus, for intact cells, it was needed to apply a high frequency (nearly 300 Hz) to produce the electroporation of the cell membrane and the increase of the extraction yield of bioactive compounds.

Martín-García et al. (2020) in their study about PEF pre-treatment to improve the extraction of phenolic compounds from brewers spent grains, also analyzed the effect of frequency (50, 100 and 150 Hz). In this study the most effective value of frequency was 50 Hz (2.5 kV·cm^{-1}) obtaining a maximum value of 99 µg·g^{-1} of phenolic compounds. It is important to consider this parameter because it determines the amount of electrical energy per unit of time applied and as observed, the lower frequency the more effective is the treatment.

4.4 Pulse duration

Pulse duration is another key variable to consider in PEF application. Usually, when pulse duration is increased, the extraction yield is also increased. However, if the pulse duration exceeds a threshold, it can produce the opposite effect. Thus, Lin et al. (2012) studied the extraction of calcium malate from eggshell treated with PEF. The eggshell was dissolved in malic acid to a concentration of 4% and then treated by PEF at the electric field strength of 15 kV·cm^{-1}, with different pulse duration (4, 8, 12, 16 and 20 µs). The authors observed that the extraction of calcium malate from the eggshell (ESCM) increased from 5.05 mg/ml to 7.05 mg/ml when applying pulses between 4 to 16 µs, respectively. However, the content of ESCM decreased when pulse duration increased from 16 to 20 µs. This result indicated the existence of an optimum pulse duration for this application. When it is compared with the results obtained for the non-treated samples, the difference in extraction of ESCM was significant, so the duration of the pulse affects the extraction yield. The conclusions of this study agree with the ones observed by Naliyadhara et al. (2022), when they observed that the number of pores in the cell membrane increases with the increase in pulse duration.

On the contrary, Maza et al. (2020) did not find significant differences between the results obtained at two different pulse durations, 3 µs and 100 µs, when carrying out polyphenol extraction during maceration/fermentation of grenache grapes (electric field strength of 4 $kV\cdot cm^{-1}$). This can lead to the conclusion that, although it is a key parameter in the extraction process, there are other parameters, such as electric field strength, which exerts the main control of the process.

<u>4.5 Solvent</u>

The type of solvent considered may affect not only the intrinsic parameters of the PEF process, mainly related with the electric conductivity, but also can appear synergistic effects between PEF and the solvent, which favors the extraction of the interesting compounds. Thus, both the ability of each solvent to solubilize the interesting compounds and the permeabilization capacity of PEF can make easier the penetration of the solvent into the cell.

In this sense, Carpentieri et al. (2022) studied the effect of PEF pretreatment on the extractability of aroma and bioactive compounds from aromatic plants and food by-products with green solvents (ethanol/water solutions and propylene glycol). The effect of PEF in the extraction of vanillin, theobromine, caffeine, linalool and limonene was confirmed by analyzing the disintegration index and permeabilization of the cell membrane. Different PEF treatments (electric field strength from 1 to 5 kV/cm and energy input from 1 to 40 kJ/kg) in ethanol/water and propylene glycol solutions were applied to the aromatic plant samples. After that, conventional extraction process in both solvents were carried out with both pretreated and control samples. When ethanol/water was used as solvent, the limonene yield obtained in PEF pretreated samples was 33% higher than the yield observed in the non-pretreated ones (control). On the other hand, when propylene glycol was considered as a solvent, no differences were found between pretreated and non-pretreated samples. Regarding the extraction of linalool, it was also higher (114%) in ethanolic extracts obtained from samples pretreated with PEF than in those obtained from non-pretreated ones. This can be explained, because PEF increased the permeabilization of cell membranes and facilitate the ethanol penetration and the dilution of non-polar compounds, such as linalool. Same effects were found in the extraction of vanillin and caffeine, with an increase of 14% and 34% respectively.

Andreou et al., (2020) studied the effect of PEF on the extraction of high-added-value compounds from olive pomace, polyphenol, and protein. For this purpose, they carried out extraction experiments without PEF using different concentrations of ethanol/water solutions (25, 50 and 70 % v/v) as solvents, being the control, the extraction carried out with 0 % of ethanol. The results indicated that the maximum extraction values of both high-added-value compounds were achieved with a 50 % of ethanol/water mixture. Afterwards, they used this concentration of ethanol for the PEF treatment (1.0 to 6.5 $kV\cdot cm^{-1}$, 0.9 to 51.1 $kJ\cdot kg^{-1}$, and 15µs pulse width) and obtained an increase of the phenolic concentration in the extract up to 91.6%. This effect can be attributed to cell alteration and permeabilization, mainly in the phospholipid bilayer of biological membranes, caused by PEF and the ethanol. This is a miscible organic solvent whose main characteristic is the penetration into the cells, which membrane is composed of a hydrophobic fraction that prevent the passage of water as a solvent.

Eing et al. (2013) tested the PEF effect on the extraction of a by-product from a microalgae, *Auxenochlorella protothecoides*. They found that when applying PEF (electric field strength of 35 kV/cm and pulse duration of 1 µs), the water-soluble component extraction was increased. However, the residual biomass predominantly contained lipids. For the extraction of lipids, a second step was needed, where ethanol was used combined with PEF. The extraction yield of lipids was improved with the use of a 70% ethanol/water solution increasing up to 9 times the percentage of lipids extracted from the algae, when compared with the non-treated sample.

In summary, it can be stated that Pulsed Electric Field (PEF) is a very attractive technique to improve extraction processes. It has many advantages and a very wide range of applications. It may be consistent with the use of green solvents and the reduction of the energy consumption. However, in order to optimize the application of this technique it has to be studied the fit of all the process variables to each particular case.

Table 2. PEF treatment application in extraction processes.

Author	**Sample**	**Extracted components**	**Operating conditions**	**Comments**
Andreou et al., 2020	Olive pomace	Polyphenols and proteins	E = 1 to 6.5 kV·cm^{-1} N =1 to 6000 pulses tp = 15 μs f = 20 Hz Solvent = 0, 25, 50 and 70 % ethanol/water U = 0 to 34 kJ·kg^{-1}	- The solvent used affected the extraction yields of each high-added-value compound. - Energy input enhanced the extraction of polyphenols and proteins.
Aouir et al., 2015	*Arthrospira Platensis*	Phycopiliproteins	E= 3 and 3.5 kV·cm^{-1} t= 0.49 and 0.54μs	- The higher the electric field applied the higher the extraction yield. - At lower electric field (2 kV·cm^{-1}) tested, the higher purity of the extracts.
Bobinaitė et al., 2015	Blueberry	Polyphenols and anthocyanins	E = 1, 3 and 5 kV·cm^{-1} f = 10 Hz U = 1, 5 and 10 kJ·kg^{-1}	- Cell disintegration index increased with intensity of PEF. - Electric field strength and energy input effects in extraction were correlated. - The extraction yield of total phenolic content and total anthocyanin content was enhanced.
Carpentieri et al., 2022	Aromatic plants and Food By-Products (Vanilla pods, cocoa beans, orange peels)	Aroma and bioactive compounds	E= 1-5 kV·cm^{-1} U= 1- 40 kJ·kg^{-1} Solvents= ethanol and propylene glycol	- Ethanol had better results in the extraction processes. - Maximum disintegration index was shown for vanilla pods at 3 kV·cm^{-1} and 20 kJ·kg^{-1}. - The highest resistance to electropermeabilization was observed in orange peel (lowest disintegration index was observed even with the use of 5 kV·cm^{-1} and 40 kJ·kg^{-1}).
Eing et al., 2013	*Auxenochlorella protothecoides*	Lipids	f = 1-4 Hz Solvent = 50, 70 and 100 % ethanol/water U = 50 to 200 kJ·kg^{-1}	- 70% ethanol/water was established as the best extraction solvent. - PEF treated extraction yield is four times higher than untreated samples.

Lin et al., 2012	Egg shell	Calcium malate	E = 5 - 30 kV·cm^{-1} tp = 4 – 20 µs	- The extraction yield of calcium malate from the eggshell increased with the duration of the pulse. - The most effective pulse duration was 16 µs.
Luengo et al., 2013	Orange peel	Polyphenols	E = 1 to 7 kV·cm^{-1} tp = 3 µs C = 200 A N = 5 to 50 pulses	- Higher values of cell disintegration index were obtained with higher electric field strengths. - Extraction yield of naringin was increased by the effect of electric field strength.
Martín-García et al., 2020	Brewers spent grains	Phenolic compounds	E= 0.5, 1.5 and 2.5 kV·cm^{-1} f= 50, 100 and 150 Hz t= 5, 10 and 15s	- Improvement in the phenolic recovery with the used of PEF as a pretreatment. - Optimal conditions: 2.5 kV·cm^{-1}, 50 Hz, and 14.5 s
Maza et al., 2020	Grenache grapes	Polyphenols	E = 0.70 to 7.80 kV·cm^{-1} N = 3 to 50 pulses tp = 3 and 100 µs f = 12-290 Hz U = 2.87 to 6.72 kJ·kg^{-1}	- Total polyphenol extraction was enhanced. - Maceration time decreased with the use of PEF treatment. - No influence of the pulse duration was found.

Segovia et al., 2015	*Borago officinallis* L. leaves	Polyphenols	E = 1, 3, 5 and 7 $kV\cdot cm^{-1}$ T = 10, 25 and 30 ºC	- Significant differences were found between the treated and control samples. - PEF treatment increased the total polyphenol content of extracts. - Cellular disintegration index increased with the electric field strength.

E= electric field strength, f= frequency, U= specific energy input, tp = pulse duration, N = number of pulses

5. ULTRASOUND APPLICATION IN EXTRACTION PROCESS

The effects of ultrasound in extraction processes can be beneficial, since they can help to intensify mass transfer, affecting both the yield and the kinetic of the process. In solid-liquid extraction operation, ultrasound can induce sponge effect and the generation of micro-channels in solids and cavitation in liquids. All these phenomena can make easier the penetration of solvent in solid matrix and the extraction of interesting compounds. However, the extent of ultrasound effects depends on process variables considered.

5.1 Power applied

The acoustic power supplied is a key parameter that affects the influence of ultrasound in extraction processes. In the literature, this parameter can be found expressed as the electric power consumed by equipment/supplied to the transducers (in some equipment, can be modulated at a percentage of the maximum power of the equipment), or as the real acoustic power applied (measured for example by calorimetry or by a hydrophone). The increase of this parameter intensifies ultrasound effects, like cavitation or microstirring, and usually enhance the extraction.

Ahmad-Qasem et al. (2013) studied the influence of this parameter in the extraction of polyphenols from olive leaves. They tested the application of different percentages of electric power of the total capacity (from 40 to 100% of 400 W) of a probe system provided with an emitter surface of 12.6 cm^2. These authors also measured the actual acoustic power applied by calorimetry, which ranged from 12.6 to 28.4 W/cm^2. They found that the greater the power supplied, the higher the extraction yield. Thus, the greater cavitation intensity produced by the ultrasound in these conditions, can generate greater effects, making the penetration of the solvent into the matrix easier and enhancing the extraction efficiency.

Da Porto et al. (2013) also reported that the ultrasound-assisted extraction yield of grape seed oils increased with the increasing of ultrasonic power applied. In its study, when the power increased from 50 to 150 W/cm^2, the extraction yield increased from 11.42% to 14.08%. This result showed that the ultrasonic power supplied has a direct effect on the extraction yield.

5.2 Frequency

In ultrasound application, frequency is the number of times that one particle passes through a fixed position by unit of time (Hz). The attenuation of the acoustic wave is very dependent of frequency, the higher the frequency the greater the attenuation. This fact can affect the intensity of cavitation and then the effects of mass transfer being at the lower frequency of ultrasound (20 kHz), the larger cavitation bubbles. This means that greater implosions can be achieved, and consequently more efficiency in the extraction processes. The effect of frequency can also be linked to the influence on the external and internal resistances that the cells have to the mass transfer (Esclapez et al., 2011). Thus, Furuki et al. (2003) observed that the more effective range of ultrasonic frequency for the extraction of phycocyanin from *Spirulina platensis* was from 20 kHz to below 40 kHz. The application of frequencies above 40 kHz let to no effect in enhancing phycocyanin extraction. In addition, they observed that the purity of phycocyanin also depended on the ultrasonic frequency, the lower the ultrasonic frequency applied the higher the purity of the extracts. So, the purity of the extracted phycocyanin can be controlled throughout the frequency of applied ultrasonic waves.

5.3 Solvent

One of the process variables that can condition the effects of ultrasound, is the solvent used. It is known that ethanol/water mixtures have been more efficient extracting components, such as phenolic compounds, than monocomponent solvent systems (Galvan D'Alessandro et al., 2012). However, this extraction capacity may not be adequate for the extraction of other types of compounds, such as fatty acids. As explained above, for extraction processes, it is important to determine the solvent to be used, since it must dissolve the component of interest. In addition, the action of ultrasound is conditioned by the characteristics of the solvent, such as viscosity or surface tension, affecting the magnitude of the ultrasonic effects such as cavitation.

Albu et al. (2004) studied the ultrasonically assisted (40 kHz; ultrasound power applied not specified) extraction efficiency of carnosic acid from rosemary (*Rosmarinus officinalis*) using butanone, ethyl acetate and ethanol as solvents at three different times (15, 30 and 45) and temperatures (25, 35 and 50 ºC). The highest extraction yield (20 mg of carnosic acid/ g) was obtained at 50 ºC with the use of butanone as solvent, in particular the extraction yield compared to the control showed an increase of 10%, being a 23% higher than the extraction with ethanol and a 19% higher than ethyl acetate. This can be due to an intensification of mass transfer arising from the collapse of cavitation bubbles near the cell walls, which forces the solvent into the cell to dissolve all the interesting components.

Jovanović et al. (2017) studied the effect of solvents with different polarity (0, 30, 50 and 70 % ethanol/water mixtures v/v) in the extraction of polyphenols from *Thymus serpyllum* L. using ultrasound-assisted techniques (Table 3). The process parameters used were a temperature of 25 ºC, power supplied of 750 W, 20 kHz of frequency and 0.3, 0.7 and 1.5 mm of particle size. The maximum total polyphenol content was obtained using a 50 % ethanol-water solvent and ultrasound. However, the difference between experiments carried out without and with ultrasound application was greater with the use of 30% ethanol as solvent. These results indicated that most of the present polyphenols in this aromatic herb had relatively high polarity. The presence of water as part of the solvent combined with ultrasound application made easier the extraction process of polyphenols. Furthermore, the use of solvent mixtures can make possible to extract both polar and non-polar polyphenols. Apart from using the correct solvent, which helps to enhance the extraction, the action of ultrasound can contribute to enhance mass transport, obtaining, in this case, an amount of total polyphenol content 17% higher than in the extracts obtained by conventional extraction.

Both et al. (2014) studied the extraction of polyphenols from black tea using two different types of solvent, ethanol-water mixture, and pure ethanol, assisted or not by ultrasonic techniques. Ultrasound was applied at a frequency of 25 kHz and a maximum input power of 150 W. The results revealed that in conventional extraction experiments, differences between both solvents appeared in the yield. With the use of ethanol and water instead of pure ethanol, the equilibrium extract can be enhanced by about 500%. Once the solvent was fixed, experiments were carried out using conventional and ultrasonic extraction, the results conclude that ultrasound leads to higher concentrations than conventional (approximately 13%).

Jovanović et al. (2017) also studied the influence of solid-solvent ratio (1:10, 1:20 and 1:30) and the application of ultrasound in the extraction of polyphenols from *Thymus serpyllum* L. These authors observed that the higher proportion of solvent versus solid produced an increase of the attenuation of the ultrasonic waves. In addition, the increase of the amount of solvent also contributed with a faster saturation of the extracting medium. The extraction yield increased from

16.87 mg/L of the control experiments (ratio 1:10) to 27.50 mg/L when using ultrasound. Therefore, there was a clear relationship between the application of ultrasound and an optimum solid-solvent ratio in which the effects of ultrasound on the cell walls and the particle size reduction were maximized, increasing the mass transfer, the diffusion, and membrane permeabilization. The use of ultrasound instead of conventional techniques (maceration) increased the extraction by 19%, with the use of the same solid-solvent ratio (from 13.7 mg/L to 16.9 mg/L).

5.4 Temperature

Since temperature affects the physical and chemical characteristics of a product and the effects of ultrasound application are linked to the product characteristics, temperature appears to have a high effect in ultrasound assisted extraction (Esclapez et al., 2011). It is well-known that the increase in the temperature enhances the extraction yield due to increases solvent diffusivity and vapor pressure and decrease of the surface tension, which reduced the energy needed to induce cavitation.

Ahmad-Qasem et al. (2013) reported results in the extraction of polyphenols from olive leaves by using power ultrasound (400W and 3.8 cm^2 emitter surface). The influence of the temperature was studied in the range of 25 to 50 ºC. Thus, the extraction yield increased at higher temperatures compared to the lower ones. This was due to the decrease of the viscosity of the liquid and the increase of the surface tension, which enhanced mass transfer. This was also observed by Zhang et al. (2010) in their study about ultrasonic-assisted extraction of flavonoids from *Prunella vulgari L.* (extraction time varied from 10 to 50 min, temperature range was 40 to 80 ºC and ethanol-solvent concentrations from 20 to 60%). The extraction of flavonoids increased when the temperature increased from 60ºC to 80ºC, being the temperature a significant parameter in the ultrasound process.

However, not always the antioxidant extraction yield is enhanced by the increase of the temperature. Rostagno et al. (2007), studying the extraction of isoflavones from soy beverages (10 to 60 ºC; 5 to 30 min) with application of ultrasound found that the amount of isoflavones extracted at the highest temperature tested (60ªC) was lower that to those obtained at lower temperature. These authors attributed this fact to of the thermal degradation of the compounds to be extracted. Therefore, it could state that the effect of the temperature is product and compound dependent (Ahmad-Qasem et al., 2013).

5.5 Particle size

Another factor to be considered in extraction operations with ultrasound assistance is the particle size. This variable has been demonstrated to significantly affect the extraction yield, the higher the particle size, the lower extraction yield. This variable was studied by Galvan D'Alessandro et al. (2012) in the extraction of polyphenols from black chokeberry. They tested five different particle diameters (d) (d < 0.5 mm, d = 0.5-1 mm, d = 1.0 – 1.4 mm, d = 1.4 – 2 mm and d > 2 mm). A decrease of the mass transfer rate, so a decrease in the extraction yield, was observed when the particle size was increased. This can be linked to the surface exposed to the ultrasonic waves, at higher surface exposure, higher ultrasonic effect. This trend is also observed without the use of the ultrasonic technique since this is due to the ratio volume-surface. But the difference is that in the extraction with ultrasound, better extraction yields were obtained in 15 minutes, than without the use of ultrasound in 60 minutes, the percentage extraction yield was increased from 29% to 42%.

It has also been considered the fact that when the particles are too small, most of the cell walls are broken by the application of ultrasound and the diffusion step in extraction will not be significant (Esclapez et al. 2011). This effect was also reported by Yeop et al. (2017) in their study about the effect of particle size and type of solvent on the extraction of gallic acid from *Labisia pumlia* by ultrasound assistance. They studied different particle diameters, ranged from 125 to 800 µm, and the results obtained showed that smaller size (125 µm) gave higher gallic acid extraction yield.

In summary, ultrasound assisted extraction technique is an interesting process to obtain high extraction yields and high valuable compounds from food (Galvan D’Alessandro et al., (2012); Jovanović et al., (2017); Ahmad-Qasem et al., (2013); da Porto et al., (2013); Both et al., (2014)). However, it is crucial to establish correctly the parameters related to this technique to obtain the highest yield and quality.

Table 3. Ultrasound application in extraction processes.

Author	Sample	Extracted components	Operating conditions	Comments
Ahmad-Qasem et al., 2013	Olive leaves (var.Serrana)	Polyphenols	T = 25, 30, 35, 40, 45, 50 ºC Solvent = 80 % ethanol/water (v/v) t = 0-15 min Power = 40, 60, 80 and 100% of 400W Emitter surface = 1.5, 3.8 and 12.6 cm^2	- The acoustic power applied had a significant influence on the final extracts after 15 min of extraction. - The best extraction yield was obtained supplying 100% of the total electric power. - The smallest emitter surface concentrated the ultrasound energy, producing an intense cavitation.
Albu et al., 2004	*Rosmarinus officinallis*	Antioxidant	T = 25, 35 and 50 ºC Solvent = ethanol, butanone, and ethyl acetate f = 20 - 40kHz	- Ethanol/water mixture provided the more effective extraction. - Ultrasound is a more effective technique when we compare it to conventional ones.
Both et al., 2014	Black tea	Polyphenols	Solvent = pure ethanol and ethanol: water (90:10) f = 25 kHz t = 30 min Power = 150 W Particle size = 0.3, 0.7 and 1.5 mm	- Water leads to higher solubility and swelling of the raw material and hence higher extraction yields. - Better extraction yields where obtained with ethanol/water solvent.
da Porto et al., 2013	Grape seeds (*Vitis vinífera L.)*	Oil and polyphenols	T = below 30 ºC Solvent = methanol f = 20 kHz t = 30 min Power = 50, 100 and 150 W	- The increase of power applied increased the yield of grape seed oil extraction. - Lower solvent consumption. - Shorter extraction time.

Furuki et al., 2003	*Spirulina platensis*	Phycocyanin	T = below 10 ºC f = 20 - 40 kHz t = 0 - 7 min	- No significant improve of extraction was found for frequencies higher than 28 kHz. - The phycocyanin concentration increased with the application time.
Galvan D'Alessandro et al., 2012	Black cherry	Polyphenols	T = 20, 40, 60 and 80 ºC Solvent = 20, 50, 80 and 95 % ethanol/water (v/v) Solid solvent ratio = 1:10, 1:20, 1;40 f = 30.8 kHz t = 0 - 250 min Power = 100 W	- The most suitable solvent for the experiment was 50 % ethanol/water mixture. - Time was not a significant parameter in the experiment. - Temperature and ultrasonic power enhanced the extraction yield of polyphenols (best combination 60ºC and 100 W). - The higher the solvent/solid ratio, the greater the extraction yield.
Jovanović et al., 2017	*Thymus serpyllum* L.	Polyphenols	Solvent = 30, 50 and 70 ethanol/water (v/v) Solid solvent ratio = 1:10, 1:20, 1:30 f = 20 kHz t = 0 - 90 min Power = 750 W Particle size = 0.3, 0.7 and 1.5 mm	- Improvement of total polyphenol content extraction was shown when the size of the particle decreased. - Extraction yield decreased as the amount of solid increased and the solvent decreased. - The total polyphenol yield was maximized at 50% ethanol.
Rostagno et al., 2007	Soy beverages	Isoflavones	Solvent= ethanol and methanol Sample/solvent ratio= 0.2:1 to 5:1 T= 10 to 60 ºC t= 5 to 30 min	- Ultrasound assisted extraction of isoflavone can be extracted in a simple and reproducible way. - Ethanol is the optimum solvent. - The optimized method was achieved with a sample/solvent ratio 0.2:1, on an ultrasound bath at 45ºC during 20 min.

Yeop et al., 2017	*Labisia pumila*	Gallic acid	Solvent= 10% ethanol/water (v/v) Solid/solvent ratio= 1:20 t= 10 min Power= 90% of the total Particle size= 125 to 900 µm	- Ultrasound application provided higher yield and the lowest particle size increased the extraction yield.
Zhang et al., 2010	*Prunella vulgaris L.*	Flavonoids	Solvent= 20 to 60% of ethanol/ water (v/v) f= 40 kHz T= 40 - 80 ºC t= 10 to 50 min	- Ultrasonic assisted extraction is an effective method for extraction. - Temperature is an influential parameter.

f= frequency, T= temperature, t= time

6. CONCLUSION

Electric fields treatments, such as MEF and PEF, or the application of ultrasound can enhance both the yield and the kinetics of the extraction of bioactive compounds from biobased materials. The MEF and PEF treatments can induce degradation of the cell membrane forming pores, mechanism known as electroporation. This effect can facilitate the mass transfer and diffusivity of the compounds towards the extracellular medium through the cell membrane. In the case of ultrasound application, effects such as induced cavitation can also produce this cellular wall degradation, improving the extraction process. In addition, other effects such as the microstirring at interfaces can reduce the external resistance to mass transfer improving the mass transport. Thus, these techniques can help the use of environmentally friendly solvents and can reduce the energy consumption.

The process variable of each technique, such as power or electrical conductivity and frequency, for example, can significantly affect the extraction process. Moreover, the conventional process variables of extraction processes such as type of solvent, size of particle or temperature, also can influence in the effect of these techniques. Finally, the properties of each food subjected to these techniques are also significant. Thus, the optimum treatment must be identified for each specific case. Therefore, it is necessary to deep in the knowledge of the application of these innovative techniques on extraction processes, to establish the parameters of each technique in the most appropriate way and find the best combination to favor the extraction performance.

6. BIBLIOGRAPHY

Ahmad-Qasem, M., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J. A., & García-Pérez, J. V. (2013). Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound. *Innovative Food Science and Emerging Technologies*, *17,* 120–129.

Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J. P., & Mason, T. J. (2004). Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrasonics Sonochemistry*, *11*(3–4), 261–265.

Andreou, V., Psarianos, M., Dimopoulos, G., Tsimogiannis, D., & Taoukis, P. (2020). Effect of pulsed electric fields and high pressure on improved recovery of high-added-value compounds from olive pomace. *Journal of Food Science*, *85*(5), 1500–1512.

Aouir, A., Amiali, M., Kirilova-Gachovska, T., Benchabane, A., & Bitam, A. (2015). The effect of Pulsed Electric Field (PEF) and Ultrasound (US) technologies on the extraction of phycopiliproteins from *Arthrospira Platensis*. *IEEE Canada International Humanitarian Technology Conference (IHTC).*

Arenas, S. (2018). Extracción de compuestos fenólicos mediante el uso de disolventes orgánicos a partir del subproducto obtenido en la elaboración de aceite de olivar virgen (alperujo). Universitat Politènica de València.

Barros, M., Salvador, R., de Francisco, A., & Piekarski, C. (2020). Mapping of research lines on circular economy practices in agriculture: From waste to energy. In *Renewable and Sustainable Energy Reviews* (Vol. 131).

Basset, A., & Vorobiev, E. (2001). *Effect of moderate electric field pulses on the diffusion coefficient of soluble substances from apple slices*. International Journal of Food Science and Technology 2002, 37,73-86.

Boonchoo, N., Tongprasan, T., Tangduangdee, C., & Asavasanti. S. (2014). Effect of ohmic pretreatment at different electrical field strengths on yield and quality of lime oil obtained from hydrodistillation. International Conference on Sustainable Global Agricultural and Food Security.

Bobinaitė, R., Pataro, G., Lamanauskas, N., Šatkauskas, S., Viškelis, P., & Ferrari, G. (2015). Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. *Journal of Food Science and Technology*, *52*(9), 5898–5905.

Both, S., Chemat, F., & Strube, J. (2014). Extraction of polyphenols from black tea - Conventional and ultrasound assisted extraction. *Ultrasonics Sonochemistry*, *21*(3), 1030–1034

Buckow, R., Baumann, P., Schroeder, S., & Knoerzer, K. (2011). Effect of dimensions and geometry of co-field and co-linear pulsed electric field treatment chambers on electric field strength and energy utilization. *Journal of Food Engineering* 105 (2011) 545-556.

Camargo, M., Alves dos Santos, C., Martins de Oliveira, A. A., & Vessoni, T. C. (2010). Ohmic heating - a review. In *Trends in Food Science and Technology* (Vol. 21, Issue 9, pp. 436–441).

Carpentieri, S., Rezek Jambrak, A., Ferrari, G., & Pataro, G. (2022). Pulsed Electric Field- Assisted extraction of aroma and bioactive compounds from aromatic plants and food by-product. *Frontiers in Nutrition.*

Chemat, F, Zill-E-Huma, & Khan, M. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry, 18*(4), 813–835.

Coelho, M., Pereira, R., Rodrigues, A.., Teixeira, J., & Pintado, M. (2019). Extraction of tomato by-products' bioactive compounds using ohmic technology. *Food and Bioproducts Processing*, *117*, 329–339.

Da Porto, C., Porretto, E., & Decorti, D. (2013). Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera L.*) seeds. *Ultrasonics Sonochemistry, 20*(4), 1076–1080.

de Oliveira, C., Giordani, D., Gurak, P., Cladera-Olivera, F., & Ferreira, L. (2015). Extraction of pectin from passion fruit peel using moderate electric field and conventional heating extraction methods. *Innovative Food Science and Emerging Technologies*, *29*, 201–208.

Eing, C., Goettel, M., Straessner, R., Gusbeth, C., & Frey, W. (2013). Pulsed electric field treatment of microalgae - Benefits for microalgae biomass processing. *IEEE Transactions on Plasma Science*, *41*(10), 2901–2907.

Esclapez, M. D., García-Pérez, J. V., Mulet, A., & Cárcel, J. A. (2011). Ultrasound-Assisted Extraction of Natural Products. In *Food Engineering Reviews* (Vol. 3, Issue 2, pp. 108–120). Universitat Politècnica de València.

Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., Ito, K-, & Nozawa, H. (2003). *Rapid and selective extraction of phycocyanin from Spirulina platensis with ultrasonic cell disruption*. Journal of Applied Physcology 15: 310-324,2003.

Galvan D'Alessandro, L., Kriaa, K., Nikov, I., & Dimitrov, K. (2012). Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and Purification Technology*, *93*, 42–47.

Gavahian, M., Chu, Y., & Sastry, S. (2018). Extraction from Food and Natural Products by Moderate Electric Field: Mechanisms, Benefits, and Potential Industrial Applications. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 17, Issue 4, pp. 1040–1052). Blackwell Publishing Inc.

Gavahian, M., & Chu, R. (2022). Ohmic heating extraction at different times, temperatures, voltages and frequencies: A new energy-saving technique for pineapple core valorization. Department of Food Science, National Pingtung University of Science and Technology, Neipu, Pingtung 91202, Taiwan.

Jaeschke, D., Menegol, T., Rech, R., Domeneghini, G., & Ferreira, L. (2016). Carotenoid and lipid extraction from *Heterochlorella luteoviridis* using moderate electric field and ethanol. *Process Biochemistry*, *51*(10), 1636–1643.

Jovanović, A., Đorđević, V., Zdunić, G., Pljevljakušić, D., Šavikin, K., Gođevac, D., & Bugarski, Branko M. (2017). Optimization of the extraction process of polyphenols from *Thymus serpyllum L.* herb using maceration, heat- and ultrasound-assisted techniques. *Separation and Purification Technology*, *179*, 369–380.

Khalaf. W, & Sastry. S. (1996). Effect of Fluid Viscosity on the Ohmic Heating Rate of Solid-Liquid Mixtures. Journal of Food Engineering (Vol. 21.)

Knirsch, M. C., Alves dos Santos, C., Martins de Oliveira, A. A., & Vessoni Penna, T. C. (2010). Ohmic heating - a review. In *Trends in Food Science and Technology* (Vol. 21, Issue 9, pp. 436–441).

Kulshrestha, S., & Sastry, S. (2002). Frequency and voltage effect on enhanced diffusion during moderate electric field (MEF) treatment. *Innovative Food Science and Emerging Technologies* 4 (2003) 189-194.

Lakkakula, N., Lima, M., & Walker, T. (2004). Rice bran stabilization and rice bran oil extraction using ohmic heating. *Bioresource Technology*, *92*(2), 157–161.

Lebovka, N. I., Shynkaryk, M. & Vorobiev, E. (2007). Moderate Electric Field of sugar beet tissues. *Byosystems Engineering* 97 (1), 47-56.

Lentacker, I., de Cock, I., Deckers, R., de Smedt, S. C., & Moonen, C. T. W. (2014). Understanding ultrasound induced sonoporation: Definitions and underlying mechanisms. In *Advanced Drug Delivery Reviews* (Vol. 72, pp. 49–64). Elsevier.

Lima, M., & Sastry, S. (1999). The effects of ohmic heating frequency on hot-air drying rate and juice yield. *Journal of Food Engineering 41* (1999) 115-119.

Lin, S., Wang, L., Jones, G., Trang, H., Yin, Y., & Liu, J.. (2012). Optimized extraction of calcium malate from eggshell treated by PEF and an absorption assessment in vitro. *International Journal of Biological Macromolecules*, *50*(5), 1327–1333.

Lozano, M. A. (2021). Aplicación de campos eléctricos moderados para la inactivación de esporas de *Aspergillus niger* en una emulsión. Universitat Politècnica de València.

Luengo, E., Álvarez, I., & Raso, J. (2013). Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innovative Food Science and Emerging Technologies*, *17*, 79–84.

Machado, A., Rocchetti, B., Mekaru, C., Martinez, J., Bezerra, R., & Rostagno, M. A. (2019) Extraction of polyphenols and antioxidants from pomegranate peel using ultrasound: influence of temperature, frequency and operation mode. International Journal of Food Science and Technology 2019, 54, 2792-2801.

Martín, E. (2018). Application of pulsed electric fields for food processing and preservation. Facultad de Veterinaria Universidad de Zaragoza.

Martín-García, B., Tylewicz, U., Verardo, V., Pasini, F., Gómez-Caravaca, A. M., Caboni, M. F., Dalla Rosa, M. (2020). Pulsed electric field (PEF) as pre-treatment to improve the phenolic compounds recovery from brewers' spent grains. *Innovative Food Science and Emerging Technologies 64(2020) 102402.*

Maza, M.A., Martínez, J. M., Delso, C., Camargo, A., Raso, J., & Álvarez, I. (2020). PEF-dependency on polyphenol extraction during maceration/fermentation of Grenache grapes. *Innovative Food Science and Emerging Technologies, 60.*

McCourtie, S. (26/05/2021). La economía circular: un modelo económico que lleva al crecimiento y al empleo sin comprometer el medio ambiente. Noticias ONU. https://news.un.org/es/story/2021/03/1490082

Moreno, J., Simpson, R., Baeza, A., Morales, J., Muñoz, C., Sastry, S., & Almonacid, S. (2012). Effect of ohmic heating and vacuum impregnation on the osmodehydration kinetics and microstructure of strawberries (cv. Camarosa). *LWT - Food Science and Technology*, *45*(2), 148–154.

Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. In *Analytica Chimica Acta* (Vol. 703, Issue 1, pp. 8–18).

Nair, G. Divya, V. Prasannan, L., Habeeba, V., Prince, M. V., & Raghavan, G. (2014). Ohmic heating as a pre-treatment in solvent extraction of rice bran. *Journal of Food Science and Technology*, *51*(10), 2692–2698.

Naliyadhara, N., Kumar, A., Girisa, S., Daimary, U., Hegde, M., & Kunnumakkara, A. B. (2022). Pulsed electric field (PEF): Avant-garde extraction escalation technology in food industry. In *Trends in Food Science and Technology* (Vol. 122, pp. 238–255). Elsevier Ltd.

Pataro, G., & Ferrari, G., (2021). Improving the extraction of juice and bioactive compounds from blueberry fruits and their by-products by application of Moderate Electric Field (MEF. *Chemical Engineering Transactions.* Vol. 87, 2021

Pare, A., Nema, A., Singh, V. K., & Mandhyan, B.L. (2014). Combined effect of ohmic heating and enzyme assisted aqueous extraction process on soy oil recovery. Journarl of Food Science and Technology, 1606-1611.

Pereira, R., Rodrigues, R., Genisheva, Z., Oliveira, H., de Freitas, V., Teixeira, J. A., Vicente, A. A. (2016). Effects of ohmic heating on extraction of food-grade phytochemicals from colored potato. *LWT – Food Science and Technology* 74 (2016) 493-503.

Pollini, L., Cossignani, L., Juan C., & Mañes, J. (2021). Extraction of phenolic compounds from fresh apple pomace by different non-conventional techniques. *Molecules*, *26*(14).

Puértolas, E., Luengo, E., Álvarez, I., & Raso, J. (2012). Improving mass transfer to soften tissues by pulsed electric fields: Fundamentals and applications. *Annual Review of Food Science and Technology, 3*(1), 263–282.

Rivas, A. (2012). Aplicación de pulsos eléctricos de alta intensidad en una bebida mezcla de zumo de naranja y leche: efectos sobre *Escherichia coli, Saccharomyces cerevisiae*, componentes nutricionales y calidad. Universitat Politècnica de València.

Rostagno, M. A., Palma, M., Barroso, C. G. (2007). Ultrasound-assisted extraction of isoflavones from soy beverages blended with fruit juices. Analytica Chimica Acta 597 (2007) 265-272.

Segovia, F. J., Luengo, E., Corral-Pérez, J. J., Raso, J., & Almajano, M. P. (2015). Improvements in the aqueous extraction of polyphenols from borage (*Borago officinalis L.*) leaves by pulsed electric fields: Pulsed electric fields (PEF) applications. *Industrial Crops and Products*, *65*, 390–396.

Sensoy, I., & Sastry, S.K (2004). Extraction Using Moderate Electric Fields. In Journal of Food Science (Vol. 69, Issue 1)

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

Torgbo, S., Sukatta, U., Kamonpatana, P., & Sukyai, P. (2022). Ohmic heating extraction and characterization of rambutan (*Nephelium lappaceum L.*) peel extract with enhanced and antifungal activity as a bioactive and functional ingredient in white bread preparation. *Food Chemistry* 382 (2022) 132332.

Varghese, K. Shiby, Pandey, M. C., Radhakrishna, K., & Bawa, A. S. (2014). Technology, applications and modelling of ohmic heating: a review. In *Journal of Food Science and Technology* (Vol. 51, Issue 10, pp. 2304–2317). Springer.

Vivanco, D., Ardiles, P., Castillo, D., & Puente, Lui. (2021). Emerging technology: Pulsed Electric Fields (PEF) for food treatment and its effect on antioxidant content. *Rev Chil Nutr 2021; 48(4): 609-619.*

Yeop, A., Sandanasamy, J., Pang, S., Abdullah, S., Yussoff, M., & Gimbun, J. (2017). The effect of particle size and solvent type on the gallic acid yield obtained from Labisia pumila by ultrasonic extraction. *MATEC Web of Conferences 111, 02008. FluidsChE 2017*

Zhang, G., He, L., & Hu, M. (2010). Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris L.* and evaluation of antioxidant activities in vitro. *Innovative Food Science and Emerging Technologies 12 (2011) 18-25.*