



## Review

## Electric field-based technologies for valorization of bioresources

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## ABSTRACT

This review provides an overview of recent research on electrotechnologies applied to the valorization of bioresources. Following a comprehensive summary of the current status of the application of well-known electric-based processing technologies, such as pulsed electric fields (PEF) and high voltage electrical discharges (HVED), the application of moderate electric fields (MEF) as an extraction or valorization technology will be considered in detail. MEF, known by its improved energy efficiency and claimed electroporation effects (allowing enhanced extraction yields), may also originate high heating rates – ohmic heating (OH) effect – allowing thermal stabilization of waste stream for other added-value applications. MEF is a simple technology that mostly makes use of green solvents (mainly water) and that can be used on functionalization of compounds of biological origin broadening their application range. The substantial increase of MEF-based plants installed in industries worldwide suggests its straightforward application for waste recovery.

## 1. Introduction

Food security and climate changes represent major concerns in the XXI century. It is estimated that, in 2012, 12.5% of the global population was undernourished, and this ratio increased to 15% in developing countries (FAO et al., 2012). Furthermore, the scarcity of resources demands for a rational use of land, energy, chemicals/fertilizers and water. Therefore, food security, climate changes, health and sustainability issues jumped into the last decades' political agenda and public consciousness.

The multi-valorization of underused bioresources such as agro-food wastes, forestry surplus, seaweeds or microalgae is therefore a desirable approach to meet the bioeconomy challenges. In this line of thought, using biomass as a sustainable renewable feedstock in biorefinery systems is crucial for the transition from a non-biodegradable fossil carbon-based economy to a bio-based economy (Ekman et al., 2013).

## 1.1. Undervalued bioresources

Numerous products can be obtained and/or valorized from different sources. Exploitable compounds or fractions may include proteins and peptides, polysaccharides or oligosaccharides, fibers, gum exudates, lipids, polyphenols, carotenoids and other secondary metabolites with highly-valued bioactivity. A full (bio)chemical and nutritional characterization and the identification of the relevant fractions for each

resource is the first step in most bioresources valorization strategies. Most of the compounds or fractions of interest are intracellular, and appropriate strategies for extraction, separation and further processing of the different exploitable fractions need to be designed to allow a financially and environmentally sustainable valorization of relevant byproducts or wastes (Fig. 1). Target applications cover different sectors such as food, feed, health, cosmetics, bioplastics, biomaterials or (bio)chemicals. The residual final fraction can feed different conversion systems of biorefineries such as fermentative processes (to produce high added-value compounds, bioethanol, bioplastics and/or energy) or thermochemical processes (pyrolysis) (ElMekawy et al., 2013).

Agro-food and forestry wastes, seaweeds and microalgae are biomass-derived resources that can be valorized in a circular bio-based economy approach. Food wastes can be divided according to their source: from vegetable commodities and products or from animal commodities and products; they can come from different stages in the food chain including agricultural production, post-harvest handling and storage, processing, distribution and consumption (Gustavsson et al., 2011). In a report from 2011, FAO has estimated that ca. of 1.3 billion ton of food losses and wastes are produced per year (Gustavsson et al., 2011), coming most of them from vegetable sources (cereals, fruits and vegetables, roots and tubers and oil crops and pulses). The dairy industry is responsible for the highest production of animal-sourced foods, followed by meat and fish industries.

Lignocellulosic residues in biorefineries and green extraction

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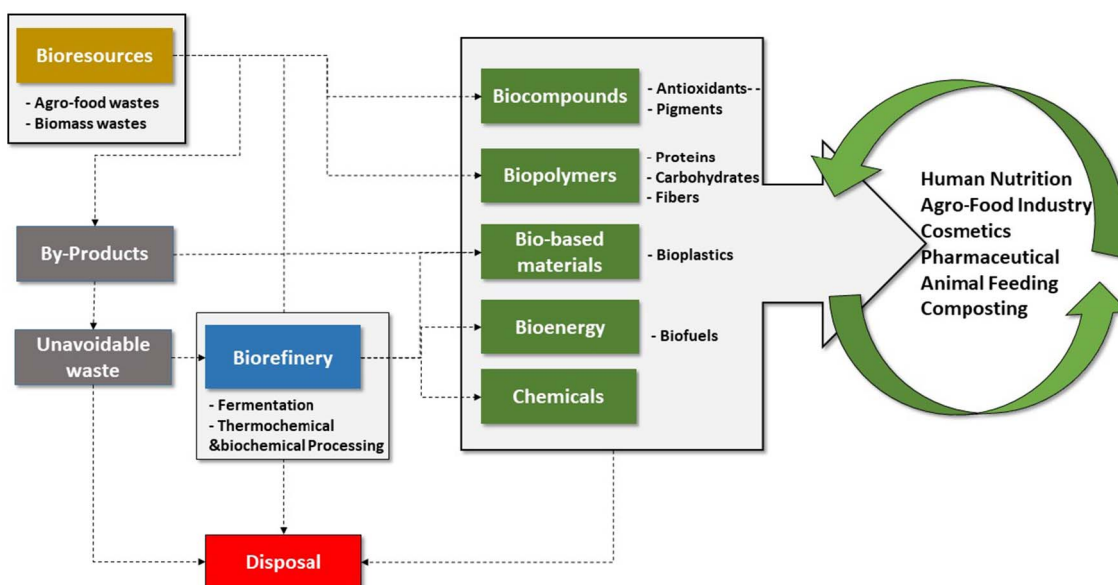


Fig. 1. Byproduct valorization chain.

methods for phytochemicals from plant-based materials have been widely studied subjects in the last years and were the aim of several recent reviews (e.g. (Ameer et al., 2017; Gillet et al., 2017)). Nevertheless, plant-based materials are also sources of other potentially exploitable compounds, including non-animal-sourced protein, enzymes, polysaccharides (such as pectin or starches), essential oils, coloring or flavoring agents and dietary fibers.

Animal-based wastes are usually rich in high quality proteins (Marcet et al., 2016). They are also good sources of proteolytic enzymes (e.g. pepsin and trypsin), collagen, gelatin, keratin, chitosan (from shrimp or crab shells), polyunsaturated fatty acids (from fish oil) and peptone (Baiano, 2014). Further specific applications include the use of amino acids, hormones, fibrinogen, albumins, insulin, among others. Lactic acid, oligosaccharides, peptides, proteins and lactulose represent major compounds present in dairy wastes (Mirabella et al., 2014).

Seaweeds are important marine bioresources being used in human consumption, hydrocolloids extraction, fertilizers, extracts for cosmetics and pharmaceuticals, biofuels and wastewater treatment (McHugh, 2003). The hydrocolloids extraction sector is the main focus of seaweed processing industry but emerging applications include production of high added-value bioactive compounds (e.g. anti-oxidant or anti-tumoral), and the use of seaweed protein for food and feed and the extraction of pigments for different applications. Besides polysaccharides and proteins, carotenoids and phenolic compounds constitute other compounds with potentially important bioactive features. Such compounds may also be obtained from residues from hydrocolloids industries and seaweed tides (such as green tides), and from sustainable seaweed production in integrated multitrophic aquaculture systems and urban coastal waters using native seaweeds. In both latter cases, seaweeds are applied as biofilters, using solar energy and the excess of nutrients to produce high amounts of new biomass while purifying the effluents (Kim et al., 2014).

The ability of microalgae to fix CO<sub>2</sub> has been proposed as a method of removing CO<sub>2</sub> from flue gases (e.g. from power plants) (Abbasi and Abbasi, 2010). Approximately half of the dry weight of microalgae biomass is carbon derived from CO<sub>2</sub> (Chisti, 2008). Considering the current carbon emissions' global market, the valorization of compounds resulting from microalgae growth appears as a straightforward solution to help coping with the algal biomass generated from CO<sub>2</sub> removal processes, while providing a renewable source of valuable compounds that does not compete with forest and does not imply water scarcity or soil erosion. Microalgae have the extra advantage of showing rapid

growth under optimal conditions. Certain species of microalgae are extremely rich in lipids (that may exceed 80% in microalgae dry weight) while others have the ability to produce high levels of carbohydrates (instead of lipids) as reserve polymers (Mussatto et al., 2010). Furthermore, they can be used as a source of food and feed proteins – single cell protein (Smetana et al., 2017), pigments for food and cosmetics or other minor valuable compounds (Matos, 2017).

### 1.2. Current status of technologies for bioresource valorization

Traditionally, solvent solid-liquid extraction (TSE) is used for most fractioning processes. The correct choice of solvents to achieve good extraction yields with a high concentration in the target compound depends on the target's solute solubility and polarity. This choice includes frequently organic compounds such as dichloromethane, ethanol, and methanol. Heat and/or agitation are usually side-by-side with TSE, both to increase the solute's solubility and increase the mass transfer rate, though minimum damage to the target compound has to be assured (e.g. avoiding oxidation and/or thermal degradation). Besides issues such as the molecular affinity between solvent and solute and mass transfer, other factors should not be overlooked such as the need for a co-solvent, environmental safety, human toxicity and financial feasibility. Traditional water or organic solvent extractions are time-consuming processes that often require high solvent and energy consumptions and generate large amounts of waste.

Issues such as growing environmental concerns and petroleum shortage as well as increasing oil price instability caused by geopolitical conflicts (causing increased costs of chemicals and energy) have boosted the search for alternative environmentally friendly extraction and fractioning methodologies, aiming at reducing energy and chemicals consumption, waste generation and operational time, while increasing overall yield, selectivity and quality of the extract. Studied alternative technologies include accelerated solvent extraction, subcritical water extraction, pulsed electric fields, supercritical fluid extraction, enzyme-assisted extraction or digestion and extrusion. The search for alternative solvents led to the use of ionic liquids, deep eutectic solvents and surfactants, as greener or more efficient options. However, downstream processing may be a problem as some of these solvents are not easily separated from the target compounds. Also, renewable solvents that can be produced from biomass such as bioethanol, terpenes, glycerol or ethyl lactate are being considered (Chemat et al., 2012). In addition, the trend is to use the least solvent possible,

**Table 1**  
Main novel and emergent electrotechnologies and its main applications in previous works.

Electric fields	Technological common names	Main applications
Pulsed	Pulsed Electric Fields (PEF)	Non-thermal inactivation of microbial cells Electroporation of cell membranes Softening tissues and peeling Extraction of thermal labile biocompounds
	Pulsed Ohmic Heating (POH) High Voltage Electric Discharges (HVED)	Thermal extraction of biocompounds Extraction of biocompounds
	Non-Pulsed	Ohmic Heating (OH)
	Moderate Electric Fields (MEF)	Separation of bioproducts Protein separation and diagnostics
	Electrofiltration Electrophoresis	

and ideally to move towards solvent-free technologies. These may include cold pressing, enzyme-assisted cold pressing, extrusion, solvent-free microwave-assisted extraction, instant controlled pressure drop or the use of electrotechnologies, such as the case of pulsed electric fields (Chemat et al., 2015) or alternating moderate electric fields combined with ohmic heating.

Process intensification towards improved sustainability, efficiency and environmental performance could be a highly beneficial approach to this kind of processes. In fact, a deep knowledge of the spatial, thermodynamic, functional and temporal domains can be used to obtain the best extraction possible for each system using four approaches, respectively: structure, energy, synergy and time (Van Gerven and Stankiewicz, 2009). In particular, instead of the conventional conductive heating with a steam boiler, a large variety of other forms and sources of energy can be considered for process intensification, including ultrasound (US), light, microwaves (MW) and electric fields (Stefanidis et al., 2014).

The focus of this review will be the on use of electrotechnologies in the extraction and bioprocessing of biocompounds or fractions from underused bioresources, aiming at their valorization. Electric fields processing is particularly interesting due to its versatility, easy scale-up, membrane electroporation effects and energetic efficiency. Though both pulsed and non-pulsed electric technologies will be addressed, special emphasis will be put in ohmic heating (OH) and its moderate electric fields (MEF), due to their more innovative character and emergent potential applications to this end.

## 2. Overview of novel and emerging electrotechnologies

To achieve the goals implicit in the concepts of circular economy and biorefinery, not only production methods and strategies have to be rethought, but also the development of new approaches and technological solutions is a fundamental requirement. Many technologies have been developed or applied in the context of bioprocessing and despite some being available for a considerable time, their industrial application has been impaired by limitations as diverse as high costs, operational problems or lack of control and knowledge of all important variables (Galanakis, 2013).

Driven by technological advances or by changes in social-economic circumstances, some of these technologies show steady growth in interest and applications, in both research and industry. These so-called “novel” or “emergent” technologies hold the potential to change the paradigm and revolutionize the bioprocessing industry (Golberg et al., 2016).

A branch of the novel and emergent bioprocess technologies is represented by the electro-technologies, which are based on the application of electric current in biomaterials with technological purposes (Kotnik et al., 2015; Lebovka et al., 2008; Sastry, 2008). The concept of applying an external electric field (EF) to promote or assist bioprocesses

has been recognized as soon as electricity became a viable technology. However, with the advances in material sciences, power generators and process variables understanding and control, it was possible to push electricity-based technologies as a viable bioprocessing alternative (Sastry, 2008). These technologies have thrived over the last 30 years and found diverse applications, as is the case of bioresources valorization (Puértolas and Barba, 2016).

The application of an EF on a biological or bio-based system will result on dissipation of heat, since the system will act as a semi-conductor. Often designated as Ohmic Heating (OH), this is explained by the Joule effect and provides a fast and homogeneous heating rate along with high energetic efficiencies (Pereira and Vicente, 2010). Other consequence of the EF presence is electroporation, as the exposure of cells to an external EF results on the formation of a trans-membrane potential. When this potential overcomes a value between 0.2 and 1 V, electroporation of the membrane is induced. The temporal nature (i.e. temporary or permanent) and extent of the permeabilization is dependent of variables such as EF intensity, exposure time, and medium composition, among others (Mahnic and Miklavc, 2014). The presence of the EF also results on charge-related phenomena, as almost all natural occurring molecules have a built-in electric charge. Electrophoresis or dielectrophoresis may occur when these molecules are subjected to continuous or non-uniform EF respectively, enabling to explore electro-kinetic phenomena for focusing, trapping or fractioning biological material (Wong et al., 2004). The application of EF may also result on the occurrence of secondary phenomena such as electrochemical reactions, shock wave formation and light emission (Lebovka et al., 2008).

The use of electrotechnologies brings advantages in different stages of bioresources valorization as they may promote stabilization of the biomaterials, endorse or enhance extraction and diffusion of compounds, assist in separation and fractioning, among others (Puértolas and Barba, 2016; Wong et al., 2004). The success of EF processing in performing one or more of these tasks will be dependent of the operational parameters and specifications applied, resulting on favoring of one or more EF-related effects and ultimately defining the technologies into subcategories (see Table 1). As a result, electrotechnologies can be classified according to type of electric flow (i.e. direct or alternating current), application in pulses or not, electric field strength (voltage applied by the section length), extension of heat deposition, among others. In this section, the major electrotechnologies applied to extraction and valorization of compounds from bioresources will be defined and addressed according to their specifications and potential applications.

### 2.1. Pulsed electric applications

The principle of pulsed electrotechnologies is the application of electric pulses, generally of high intensity, for short periods of time

(Mahnic and Miklavc, 2014). Following these principles, techniques such as High Voltage Electric Discharge (HVED), Pulsed Electric Fields (PEF) and Pulsed Ohmic Heating (POH) have been applied on the bioresources valorization (Vorobiev & Lebovka, 2016).

### 2.1.1. High voltage electrical discharges

When a direct high voltage, high current pulse is applied into a liquid medium, it results on a sudden energy release accompanied by the formation of a plasma channel. Consequently, secondary effects such as shock waves, cavitation, light emission, radical generation will occur as well (Boussetta and Vorobiev, 2014). HVED promotes heat dissipation and electroporation; however, the secondary effects resulting from the high-energy release will have predominance over EF effects (Puértolas and Barba, 2016). High levels of cellular damage or cellular disintegration can be achieved with this technique, placing it as an interesting alternative to cell inactivation and extraction of intracellular compounds. On the down side, the nonspecific extraction and release of all cellular material may impose operational problems (Boussetta and Vorobiev, 2014). The demand of technological solutions such as high-energy pulse generators, resistance of the materials to endure and contain the process and operation limitations (such as operation only in batch mode) are the main drawbacks of the method (Boussetta and Vorobiev, 2014; Liu et al., 2011; Puértolas and Barba, 2016).

### 2.1.2. Pulsed electric fields

In PEF applications, high voltages (kV range) are applied in pulses of short duration (nano or micro-seconds) with the main objective of causing electroporation (Kotnik et al., 2015). Operational parameters define the efficiency of the technique, being the EF strength commonly described as the most relevant (Raso et al., 2000). By increasing the EF strength, the electroporation effect can move from the enlargement of existing pores to the formation of new ones with temporary or permanent character, even affecting intracellular structures, and leading to cell lysis – electroporation. Nonetheless, other factors are relevant to the success and efficiency of the technique, such as wave shape, number and duration of pulses, temperature, product and media characteristics (Mahnic and Miklavc, 2014). Pulse duration and number of pulses are usually addressed as independent variables, but their conjugation defines the effective treatment time and according to some authors, they are, along with EF strength, the defining factor of PEF efficiency (Raso et al., 2000). Most of the actual PEF systems operate under square wave and alternate directional pulses. Square pulses are more effective and energetically more efficient, and by alternating the polarity operational problems such as polarization next to the electrodes, electrolytic reactions and electrode erosion, as well as medium contaminations with metal release are reduced (Elsayed and Mohammed, 2016).

PEF is conventionally addressed as non-thermal process despite some heat deposition being always present, resulting in temperature increase to significant levels. When necessary, the process is generally kept at temperatures below the necessary to cause enzymatic and microbial inactivation (Golberg et al., 2016). More recently, a trend to conjugate thermal effects and electric pulsed applications through Pulsed Ohmic Heating (POH) treatments has been gaining expression, especially in extraction processes. It has been reported that the conjugation of moderate or high temperatures with the electroporation effects can enhance extraction processes (Parniakov et al., 2016; Parniakov et al., 2014; Puértolas and Barba, 2016). In fact, significant work has been developed in POH where low EF strength pulses of longer duration have demonstrated advantageous on the pre-treatment and extraction of biological materials (Pereira et al., 2016a; Praporscic et al., 2005; Saberian et al., 2017a).

The full implementation of PEF processes is still impaired by limitations as the cost of pulse generators, lack of well standardized protocols, reliability and duration of the systems and bulk capacity (Golberg et al., 2016; Puértolas & Barba, 2016).

## 2.2. Non-pulsed applications

Non-pulsed applications are techniques where the electric current flows on a unidirectional flow (DC) or periodically reverses direction (AC) without interruption for a significant period of time. Despite DC techniques being relevant in bioprocess applications, particularly separation techniques such as electrophoresis, electrofiltration or cross-flow electrofiltration, the focus of this work is on extractions and functionalization of bioresources. Therefore, DC process will not be further addressed.

Applications involving AC usually fall under the specification of Moderate Electric Fields (MEF) where a low EF (generally between 1 and 1000 V) and defined wave shape (typically sinusoidal or square) is applied (Sastry, 2008; Varghese et al., 2012). Electric frequency is a relevant parameter in these processes, dictating efficiencies and affecting process reliability. Under low frequencies, electrochemical reactions may be an issue as they can result in the formation of radical species, corrosion and erosion of the electrodes. The use of frequencies above 15–20 kHz eliminates these problems as electrochemical reactions are completely eradicated (Pataro et al., 2014).

MEF and OH terms are often used in an interchangeable way but it is important to adopt a clear definition. OH will always be a side effect of the application of electric fields on a semi-conductive material: if the medium where MEF is applied has enough conductivity and the process takes place for sufficient time, significant heat deposition will take place through the Joule effect, thus occurring OH. For the purposes of this article OH will be mainly referred to thermal processing, while MEF will be used to give emphasis on electrical effects either when thermal aspects are attenuated or compared in a similar basis. OH resulted possibly in the most successful case of an electrotechnology with industrial application, being significantly widespread and commercially available throughout the food industry as a pasteurization technology (Jaeger et al., 2016). However, under the MEF field range, permeabilization and extraction enhancement processes are still relevant (Lebovka et al., 2005; Pereira et al., 2016a; Sensoy and Sastry, 2004). Processes as the mentioned POH or pure MEF processes, either with OH or without association of thermal effects, are gaining expression on reported applications about the valorization of bioresources. Though PEF is still the most referred technology in the literature for extraction purposes, the less demanding operational conditions of MEF, as well as the associated heating features, electric generators, electrode materials and control systems may contribute to a facilitated implementation of this technology, compared with the more challenging high energy pulsed applications (Fig. 2).

## 3. Extraction of biocompounds using electrotechnologies

### 3.1. Pulsed electric fields and high voltage electrical discharges

In the last decades electrotechnologies have been used for the extraction of biocompounds from different raw materials, mainly agro-industrial wastes (see Table 2). Pulsed electric field (PEF) and high voltage electrical discharge (HVED) are the most referred electrotechnologies used for the extraction of compounds with added value.

In particular, PEF was successfully used in different matrices to improve extraction processes of added-value compounds like pectin, polyphenols and anthocyanins.

The residues from the wine industry are largely studied, as they are known to be rich in polyphenols. Boussetta and co-authors did extensive work on the reutilization of grape pomace for extraction of polyphenols, using different electrotechnologies, concluding that PEF used for extraction of polyphenols from whole or ground grape seeds increases the yield of extraction (e.g. Boussetta et al., 2012a). Moreover, it was concluded that a mixture of ethanol and water is more efficient during PEF extraction, compared to the solvent alone (i.e. only water or only ethanol). Those authors concluded that cell membrane

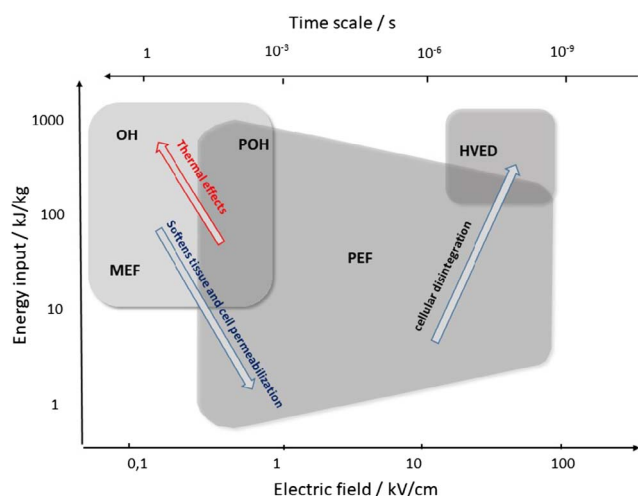


Fig. 2. Electrical pulsed and non-pulsed protocols for extraction procedures; moderate electric fields (MEF), ohmic heating (OH), pulsed electric fields (PEF), high voltage electric discharges (HVED); pulsed ohmic heating (POH).

damage was mostly a result from the use of ethanol solution together with PEF and that the extraction process is improved when the electric field strength is increased up to 20 kV/cm and the temperature is higher (50 °C). Puértolas et al. (2010) used PEF for the extraction of anthocyanins and phenols during the maceration step of wine production at pilot-plant scale, running in continuous. PEF treatments increased the extraction of polyphenols and anthocyanins during the maceration step, achieving the desired concentrations of these compounds in half of the time compared to the control process.

PEF were also applied in the extraction of compounds from other vegetable sources, including polyphenols from fresh tea leaves (Zderic and Zondervan, 2016) or betanine (a pigment) from red beetroot (López et al., 2009), also with significant reductions in the extraction time (e.g. up to five times for betanine when compared to non PEF-treated samples). The amount of extracted polyphenols depended not only on the settings of the PEF treatment (electric fields and total treatment times), but also on the relaxation time between pulses (Zderic and Zondervan, 2016).

In the case of microalgae cells, PEF was applied in the extraction of protein and carbohydrates with disappointing results: the electroporation effect was not sufficient to release a substantial amount of intracellular proteins. PEF treatments, even when combined with temperature, are not able to disintegrate sufficiently the resistant algal cells and to release protein and carbohydrates in yields comparable to bead milling, at least with the used experimental conditions (Lam et al., 2017; Postma et al., 2016).

HVED was also used in different extraction applications and polyphenol extraction from grape pomace and other winemaking residues (e.g. skins, stems, seeds or vine shoots) was addressed by different authors (e.g., Boussetta et al., 2011; Boussetta et al., 2012a; Boussetta et al., 2012b; Liu et al., 2011; Rajha et al., 2015), with significant improvements in both extraction time and amount of extracts. The effects of the energy input, the electrode distance gap, liquid-to-solid ratio, extraction time and temperature, type of solvent are variables that can be considered. The extraction yield generally increased with the number of discharges. In the case of vine shoots, the mechanical, electrical and chemical effects of HVED over polyphenols extraction from vine shoots with different initial specific surface areas were studied by Rajha et al. (2015). Different mechanical effects and high energy inputs (up to 609.5 kJ/kg) of HVED had no influence over the polyphenol content of the extracts. Even though the high-energy inputs induced higher ozone production, there was no apparent degradation of the polyphenols. This was explained with the high polyphenol content

of the vine shoot extracts and their capability of scavenging free radicals.

HVED was applied not only in laboratory scale but also at pilot scale. Boussetta et al. (2012b) compared the PEF treatment between a 1 L scale equipment and a 35 L pilot scale equipment. In both pilot and laboratory scales the use of electrical discharges increased the extracted polyphenol content about 7 times, when compared with the control. However, it was concluded that the energy output has a limit beyond which the polyphenol extraction decreases and this has to be considered for scale-up purposes. For all the materials tested, this energy limit was smaller for the laboratory scale than for the pilot scale, but results varied in accordance with the resistance of the material used for the extraction process. For example, grape stems are constituted of lignocellulosic material that makes them more resistant to electrical discharges, compared to grape skins.

These two types of electrotechnologies are frequently studied simultaneously in a PEF vs HVED approach (Barba et al., 2015a; Boussetta et al., 2012a; Carbonell-Capella et al., 2017; Grimi et al., 2014; Parniakov et al., 2016; Parniakov et al., 2014; Parniakov et al., 2015; Sarkis et al., 2015a; Sarkis et al., 2015b). Again, fruit and wine making residues are among the most studied sources of extracts. For instance, besides grape and wine making residues, papaya peels and seeds, as well as mango peels and blueberries were studied for the extraction of nutritionally valuable and antioxidant compounds (Barba et al., 2015a; Parniakov et al., 2016; Parniakov et al., 2014; Parniakov et al., 2015). PEF and HVED were used for the extraction of proteins, polyphenols, anthocyanins and carbohydrates. A comparison between PEF and HVED allowed concluding that the best extraction efficiency was achieved generally when HVED was used, except for the extraction of anthocyanin from blueberries. However, the use of HVED has limitations: the electrical discharges may produce chemical products from electrolysis and free reactive radicals, which can reduce the beneficial properties of the nutritionally valuable and antioxidant compounds. Furthermore, colloidal stability may also be reduced. Nevertheless, when HVED was used for pectin recovery from sugar beet pulp (Almohammed et al., 2017), for instance, no significant changes in functional groups and chemical composition were detected.

In the case of blueberries, Barba et al. (2015b) confirmed the capacity of PEF to achieve a selective extraction of different soluble biomolecules, proposing the use of multi-stage assisted extractions, such as PEF + HVED or PEF + supplementary extraction + HVED. The first step would extract sensitive compounds (such as anthocyanins) and subsequent steps would extract more resistant compounds. Multi-stage assisted extraction was also proposed to obtain extracts from *Stevia rebaudiana*, rich not only in steviol glycosides but also in phenolic compounds (Barba et al., 2015b). It was demonstrated once again that HVED improved the extraction of polyphenols, especially caffeic and chlorogenic acids.

Another case where PEF gave better results than HVED was in the extractions of stevioside, rebaudioside A and chlorophyll *b* from stevia leaves (Carbonell-Capella et al., 2017). Nevertheless HVED was still more efficient in the extraction of phenolic compounds and chlorophyll *a*. The treatments with PEF had to be combined with ethanol to achieve comparable performance. Ethanol will allow enhanced solubilization of the membrane bilayers, thus increasing the total phenolic content in the extracts. Finally, the authors concluded that despite the good results showed by application of HVED technology, more investigation is needed before a scale-up of the process in the industry can be achieved.

PEF and HVED can also be applied as a pre-treatment of oil extraction. Sarkis et al. (2015b) used PEF and HVED as a pretreatment of oil extraction from sesame seeds with the goal of damaging sesame seeds cells before oil extraction by pressing. PEF and HVED treatments increased the oil yield by 4.9% and 22.4%, respectively, as compared to the control sample. However, when HVED was applied considerable amounts of oil were lost in the water used for the electrical treatment. It is important to mention that the use of electrotechnologies did not

**Table 2**

Main results published on the application of electrotechnologies to improve the extraction processes of valuable compounds.

	Matrix	Extracted compounds	Optimum extraction parameters			Reference	
			Electrical	Time/ Temperature	Solvent		
Ohmic heating (OH)	Gac aril	Essential oil Carotenoids	20 kV; 50 Hz-1 MHz	n.a. 50 °C	Hexane	Aamir and Jittanit, 2017	
	<i>Prangos ferulacea</i> <i>Lindle</i>	Essential oil Terpenic compounds	120 V; 50 Hz	73 min; ≈99 °C	Water (hydrodistillation)	Damyeh and Niakousari, 2016	
	<i>Pulicaria undulata</i>	Essential oil Terpenic compounds	220 V; 50 Hz	61 min; ≈99 °C	Water (hydrodistillation)	Damyeh and Niakousari, 2017	
	<i>Myrtus communis</i> <i>Thymus vulgaris</i> L.	Essential oil Essential oil	220 V; 50 Hz 220 V; 50 Hz	≈26 min; ≈99 °C ≈24 min; ≈99 °C	Water (hydrodistillation) Water + 1% NaCl (hydrodistillation)	Gavahian et al., 2013 Gavahian et al., 2012	
	Peppermint	Essential oil	220 V; 50 Hz	≈20 min; ≈99 °C	Water + 1% NaCl (hydrodistillation)	Gavahian et al., 2015	
	Shirazi thyme	Essential oil	220 V; 50 Hz	≈32 min; ≈99 °C	Water + 1% NaCl (hydrodistillation)	Gavahian et al., 2011	
	Oregano	Essential oil	220 V; 50 Hz	31 min; ≈99 °C	Water + 2.85% NaCl (hydrodistillation)	Hashemi et al., 2017	
	Jerusalem artichoke tuber	Inulin	200 V; 20 kHz	30 min; 75 °C	Water	Khuenpet et al., 2017	
	Black rice bran	Anthocyanins	50–200 V/cm	n.a.; 105 °C	Water	Loypimai et al., 2015	
	Colored potato	Polyphenols	25 kHz; 15 V/cm	10 min; 90 °C	Water + KCL	Pereira et al., 2016	
	Orange juice	Anthocyanins Pectin	50 Hz; 15 V/cm	30 min; 90 °C	Water	Saberian et al., 2017	
	Pulsed electric field (PEF)	Grape pomace	Polyphenols Anthocyanins	40 kV; 0.5 Hz; 13.3 kV/ cm; 0–564 kJ/kg; – pulses (10 μs)	n.a.; 22 °C	Water	Barba et al., 2015b
		Blueberries	Polyphenols Anthocyanins	1–5 kV; 10 Hz; 1–10 kJ/ kg; – pulses (20 μs)	n.a.; 20–23 °C	–	Bobinaité et al., 2015
Tomato		Carotenoids	3.8 kV; 0.33 Hz; 600 pulses (350 μs of total pulses)	n.a.; 40–45 °C	–	Bot et al., 2018	
Norway spruce Bark		Polyphenols	40 kV; 0.5 Hz; 20 kV/cm; 200 pulses (10 μs)	10 min; 20 °C	Water + 0.01 M NaOH	Bouras et al., 2016	
Grape seeds		Polyphenols	40 kV; 0.33 Hz; 20 kV/cm; 400 pulses (10 μs)	n.a.; 50 °C	50% ethanol	Boussetta et al., 2013	
Stevia		Steviol glycosides Polyphenols Flavonoids Chlorophylls Carotenoids	40 kV; 0.5 Hz; 20 kV/cm; 178 kJ/kg; 200 pulses (10 μs)	n.a.; 50 °C	50% ethanol	Carbonell-Capella et al., 2017	
Spearmints		Polyphenols	20 mV; 100 kHz; 3 kV/cm; 99 pulses (10 μs)	n.a.	Mannitol solution (followed by 80% ethanol extraction)	Fincan, 2015	
Potato peels		Steroidal alkaloids	10 Hz; 0.75 kV/cm; 200 pulses (3 μs)	n.a.; 13–16 °C	Methanol	Hossain et al., 2015	
Red prickly pear		Colorants	40 kV; 0.5 Hz; 20 kV/cm; 50 pulses (10 μs)	n.a.; 20 °C	Water (followed by 1 h water extraction)	Koubaa et al., 2016	
Microalgae <i>Chlorella vulgaris</i>		Proteins	20 kV/cm; – pulses (2 μs)	n.a.; 20 °C	Water	Lam et al., 2017	
Apple pomace		Polyphenols	1 Hz; 3 kV/cm; 3.0 kJ/kg; 500 μs of total pulses	n.a.; 25 °C	Water	Lohani and Muthukumarappan, 2016	
Sorghum flour		Polyphenols	1 Hz; 2 kV/cm; 6.96 kJ/ kg; 875 μs of total pulses	n.a.; 25 °C	Water	Lohani and Muthukumarappan, 2016	
Red beet		Betanine	1 Hz; 7 kV/cm; 2.5 kJ/kg; 5 pulses (2 μs)	n.a.; 30 °C	Mcllvaine buffer	López et al., 2009	
			1 Hz; 6 kV/cm; 50 pulses (3 μs)	n.a.	n.a.	Luengo et al. 2016	
Orange peels		Polyphenols	1 Hz; 7 kV/cm; 0.06–3.77 kJ/kg; 20 pulses (3 μs)	n.a.	n.a.	Luengo et al. 2013	
Papaya peels		Polyphenols Proteins Carbohydrates	40 kV; 13.3 kV/cm; 400 pulses (10 μs)	n.a.; 35 °C	n.a.; 35 °C	Parniakov et al., 2014	
Papaya seeds		Polyphenols Carbohydrates Isothiocyanates	40 kV; 13.3 kV/cm; 300 pulses (8.3 μs)	n.a.	n.a.	Parniakov et al., 2015	
Microalgae <i>Nannochloropsis</i>		Polyphenols Proteins Carotenoids Carbohydrates	40 kV; 20 kV/cm; 400 pulses (10 μs)	n.a.; 20–30 °C	Water	Parniakov et al., 2015	
Rosé wines		Anthocyanins		n.a.; 4 °C	n.a.	Puértolas et al., 2011	

(continued on next page)

Table 2 (continued)

	Matrix	Extracted compounds	Optimum extraction parameters			Reference
			Electrical	Time/ Temperature	Solvent	
	Purple-fleshed potato	Anthocyanins	30 kV; 5 kV/cm; 122 Hz; 3.67 kJ/kg; 50 pulses (3 µs)	n.a.	Water and ethanol	Puértolas et al., 2013
	Red wine	Polyphenols	30 kV; 3.4 kV/cm; 1 Hz; 8.92 kJ/kg; 35 pulses (3 µs)	n.a.	30% ethanol	Saldana et al., 2017
	Sesame seeds	Polyphenols	40 kV; 0.5 Hz; 20 kV/cm; 40 kJ/kg	n.a.; 50 °C	Water	Sarkis et al., 2015
	Sesame cake	Proteins	40 kV; 0.5 Hz; 13.3 kV/ cm; 83 kJ/kg; 100 pulses (10 µs)	20 min; 60 °C (40 °C proteins)	10% ethanol (50% for lignans)	Sarkis et al., 2015b
	Borage	Lignans	30 kV; 300 Hz; 5 kV/cm; 6.18 kJ/kg; 50 pulses (3 µs)	n.a.; 40 °C	Acidic water	Segovia et al., 2014
	Button mushroom	Polyphenols	30 kV; 1 Hz; 38.4 kV/cm; 136 pulses (2 µs)	n.a.; 20 °C	Water	Xue and Farid, 2015
	Bone	Proteins	70 kV/cm; 12 pulses (24 µs)	n.a.; Room temperature	1.25% citric acid	Yin and He, 2008
	Rapeseed	Calcium	400 V; 0.5 kHz; 5 kV/cm (20 kV/cm for proteins); 200 pulses (10 µs)	20 min; 50 °C (20 °C proteins)	75% ethanol (water for proteins)	Yu et al., 2015
	Tea	Proteins	1.1 kV/cm; 50 pulses (100 µs)	n.a.	Water	Zderic and Zondervan, 2016
Moderate electric field (MEF)	Microalgae <i>Heterochlorella luteoviridis</i>	Carotenoids Lipids	180 V; 60 Hz	10 min; 35 °C	75% ethanol	Jaeschke et al., 2016
	Passion fruit peel	Pectin	100 V; 60 Hz	15 min; 45 °C	Acidic water	Oliveira et al., 2015
High voltage electrical discharge (HVED)	Grape seeds	Polyphenols	40 kV; 300 pulses (10 µs); 40 kV; 0.33 Hz; 100 pulses (10 µs)	n.a.; 50 °C n.a.; 50 °C	Water 50% ethanol	Liu et al., 2011 Boussetta et al., 2013
	Grape pomace	Polyphenols	40 kV; 0.5 Hz; 80 pulses (10 µs)	60 min; 60 °C	Water	Boussetta et al., 2009
		Polyphenols	40 kV; 80 kJ/kg	30 min; 60 °C	30% ethanol	Boussetta et al., 2011
		Anthocyanins	40 kV; 0.5 Hz; 280 kJ/kg	n.a.; 22 °C	Water	Barba et al., 2015b
	Sugar beet pulp	Pectin	40 kV; 0.5 Hz; 76.2 kJ/kg; 100 pulses (10 µs)	60 min; 90 °C	Water	Almohammed et al., 2017
	Flaxseed cake	Polyphenols Lignans	40 kV	60 min; 40 °C	25% ethanol	Boussetta et al., 2013
	Rapeseed	Lignin	0.5 Hz; 800 kJ/kg	80 min; 200 °C	65% ethanol	Brahim et al., 2017
	Olive kernel	Polyphenols	40 kV; 0.5 Hz; 66 kJ/kg	n.a.; 25 °C	50% ethanol	Rosello-Soto et al., 2015
	Papaya peel	Proteins	40 kV; 35 kJ/kg	272 min; 50 °C	n.a.	Parniakov et al., 2014
	Vine shots	Carbohydrates	40 kV; 0.5 Hz; 609.5 kJ/ kg; 100 pulses (10 µs)	n.a.; 50 °C	Water	Rajha et al., 2015
	Sesame seeds	Polyphenols	40 kV; 0.5 Hz; 160 kJ/kg	n.a.; 50 °C	Water	Sarkis et al., 2015
		Essential oil				
		Polyphenols				
		Proteins				
	Sesame cake	Polyphenols	40 kV; 0.5 Hz; 83 kJ/kg; 100 pulses (10 µs)	20 min; 60 °C (40 °C proteins)	10% ethanol (50% for lignans)	Sarkis et al., 2015b
		Proteins				
		Lignans				
	Pomegranate peel	Polyphenols	20 kV; 1000 Hz; pulses (2 µs)	30 min; 70 °C	Water	Xi et al., 2017
	Stevia	Steviol glycosides	40 kV; 0.5 Hz; 178 kJ/kg; 200 pulses (10 µs)	n.a.; 50 °C	Water	Carbonell-Capella et al., 2017
		Polyphenols				
		Flavonoids				
		Chlorophylls				
		Carotenoids				

n.a., not available.

decrease the quality of the produced oil. PEF and HVED pretreatments were also applied to the extraction of compounds from the remaining sesame cake was rich in proteins and polyphenols (Sarkis et al., 2015a). The conclusions of the work demonstrated that PEF and HVED increased the extraction yields of polyphenols, lignans and protein, also

allowing a reduction in the amount of ethanol used as a solvent for polyphenol extraction.

In the application of PEF and HVED in extraction of intracellular components from microalgae (Grimi et al., 2014), these electro-technologies allowed the selective extraction of water-soluble ionic

components, proteins, microelements, and organic compounds of small molecular weight. They were however ineffective in the extraction of pigments (e.g., chlorophylls or carotenoids).

In general, HVED technology is more effective in the extraction of compounds than PEF technology, as the use of HVED causes shock waves and cavitation, resulting in mechanical damage of the material and disintegration of the cell walls. Conversely, when HVED is used there is a production of oxidizing species (like ozone) that are responsible for a decrease of the polyphenols content (Boussetta et al., 2012a; Liu et al., 2011). These effects must be balanced when choosing the most adequate technology for processing.

### 3.2. Moderate electric fields and ohmic heating

In the last few years there was an increased interest in using moderate electric fields (MEF) and the corresponding ohmic heating (OH) for extraction processes. OH presents high heating rates with a precise temperature control allowing mild processing and preserving nutritional, functional and structural properties. Heat is generated inside the material to be heated (Joule effect), the heating process does not depend on heat transfer between phases and interfaces, allowing uniform heating and an extremely rapid heating rate. Furthermore, it also allows heating of large particulates and fluids at comparable rates, as long as their conductivities remain similar. Many studies also suggest that the used MEF have a significant effect on the cell wall permeabilization (Kusnadi and Sastry, 2012). The process has high energy conversion efficiencies resulting in lower operational costs and in a more environmentally-friendly system (Pereira and Vicente, 2010).

As for pulsed electrotechnologies, extraction of anthocyanins from different sources is the most common application of OH as an extraction technology. OH was used in black rice bran with the final goal of preparing a natural food colorant (Loypimai et al., 2015). The treatment with OH increased the anthocyanins content in the extracts four-fold. Extracts obtained with OH contained also the highest amounts of bioactive compounds ( $\alpha$ -tocopherol and  $\gamma$ -oryzanol). However, electric conductivity was a critical issue that needed to be adjusted, as it had a low initial value due to the high levels of fat. Pereira and co-authors (Pereira et al., 2016a), studied the effect of OH and MEF intensity in the extraction of polyphenols and anthocyanins from colored potato. Different operational parameters were considered, such as electric field strength, temperature and process time on the extraction yields. Increased extraction yields were registered at 20 V/cm and heating (90 °C). At electric field values above a critical value (20 V/cm, in this case), degradation of the anthocyanins occurred. However, when an electric field above 20 V/cm was combined with temperatures above 70 °C, an increase of polyphenol extraction yield was noticed. As this increase in polyphenol yield matches the electrical field at which the anthocyanins yield decrease, the authors suggested that this is the point of possible degradation of anthocyanins to its constituent phenolic acids. The previous results coincided with the results published by other authors with other materials. Sarkis et al., 2013 reported the anthocyanin degradation during conventional and OH extractions of blueberry pulp. The degradation of anthocyanins depended on the voltage used and on the solids content. It was concluded that when lower voltage levels were used, the degradation can be lower to that obtained during conventional heating. The use of a high voltage level led to higher anthocyanin degradation. Nevertheless the use of high-temperature short-time treatments can be combined with electric fields, with a good extraction yield of anthocyanins (up to 85%) without affecting their quality profile (Pereira et al., 2016a).

OH has been used for the extraction of essential and fatty oils (Aamir and Jittanit, 2017; Gavahian et al., 2015; Gavahian et al., 2012; Gavahian et al., 2013; Gavahian et al., 2011; Hashemi et al., 2017; Nair et al., 2014) or pectins (Saberian et al., 2017b). In all works it was concluded that the OH is the greenest technology for the extraction in terms of energy consumption in comparison to traditional techniques

(distillation, hydrodistillation or traditional heating). The use of OH generally reduced the extraction time and energy consumption, though extraction times were sometimes similar to those achieved with conventional methods.

The application of OH was not always successful. For instance, the achieved extraction yields of inulin from Jerusalem artichoke tuber powder were lower than the conventional heating process (Khuenpet et al., 2017), though the energy efficiency claim is still valid.

Application of MEF up to 1000 V/cm, may be achieved at sub-lethal temperatures (< 45 °C), thus without significant ohmic heating effect. According to the existing literature there are only few works reporting the use of MEF for extraction of added-value compounds. MEF was used for the extraction of lipids and carotenoids from microalgae (Jaeschke et al., 2016). The results demonstrated that carotenoid extraction was affected by both MEF and ethanol concentration, while lipid extraction was only affected by ethanol concentration. Moreover, analyses of the extract showed that the xanthophylls all-*trans*-lutein and all-*trans*-zeaxanthin were the major carotenoids in the extracts.

MEF was also used for pectin extraction from passion fruit (Oliveira et al., 2015). Even though the extraction yield of MEF was lower than that of the traditional extraction, the obtained pectin using different extraction methods had also similar values of galacturonic acid and esterification degree.

Though the potential is evident, MEF and OH have only marginally been applied to extract compounds mainly from vegetable tissues, including polysaccharides, essential oils and polyphenols, sugar from sugar beets, potato starch and fruit juice expression (Aamir and Jittanit, 2017; Gavahian et al., 2011; Praporscic et al., 2005; Praporscic et al., 2006; Saberian et al., 2017b; Seidi Damyeh and Niakousari, 2017; Zhu et al., 2015). Extraction procedures have been established case by case, frequently in an empirical way, and the establishment of a correlation between the chemical properties of the extracts e.g. with the intensity, frequency and other parameters associated with the application of the electric field is highly desirable. Electroporation effects under MEF are still controversial once cell lysis may also result from thermal permeabilization of the membranes due to local heating, thus more fundamental research about non-thermal effects of MEF is also needed. Nevertheless, even in the cases where no improvement in extraction yields is observed, OH will have higher energetic efficiency, which have a massive importance in thermal processing. Currently, the number of MEF based plants installed worldwide for thermal processing of foods (commonly designated by Ohmic Heating Technology) is increasing, thus it is expected that industrial application of MEF aiming at extraction for waste recovery will be straightforward and, thus, extensively applied as soon as its advantages are perceived as such by the industry.

## 4. Ohmic heating and moderate electric fields in the valorization of biomass-based by-products

Biomass-based by-products, and in particular agro-food and forestry wastes and surplus, represent a rich supply of valuable nutrients and functional biomolecules bringing together the potential needed to be used as raw material for the development of new food products, and being thus kept within the food supply chain for human nutrition (Raak et al., 2017), or to be incorporated in other value chains such as cosmetics, pharmaceuticals or bioplastics. Successful re-utilization of these streams and extractable biomolecules will always require a judicious intervention of strategies and processing technologies for safety and functional enhancement (Aspevik et al., 2017; Williams et al., 2015).

During the last decade, among the several electro-technologies available, OH is establishing a solid foothold in several applications in bioprocesses with regard to the microbiological safety and enzymatic activation/inactivation as well as bringing new insights to the functionalization of biological products. Table 3 is intended to give a comprehensive overview about the effects of OH and MEF processing



**Table 3**  
Overview of the effects of OH and MEF on functional and valorization aspects of biomacromolecules.

Matrix	Processing conditions MEF/V/cm	Frequency/Hz	Temperature/°C	Time/min	Effects	Reference
<i>Biomacromolecules</i>						
Proteins						
Whey protein Isolate	6–12	25,000	90	5	- Less protein aggregation (increased solubility)- Protein aggregates with different morphologies - Potential to encapsulation of bioactive ingredients (fibrillar appearance) - Production of stable hydrogels	Pereira et al., 2016
Whey protein isolate	10	25,000	90	5	- Higher capability to incorporate Fe <sup>2+</sup> , within protein network- Potential to transport/delivery of bioactive or functional biomolecules (pharmaceutical applications) - Less protein aggregation - Impact on rheological and microstructural behavior (weaker gel structure) of cold gel-like emulsions of lactoferrin	Pereira et al., 2017 de Figueiredo Furtado et al.
Lactoferrin	6–20	25,000	90	30	-Reduced proteolysis -Increased disulfide crosslinking -Improved water holding capacity	Tadpichayangkoon et al., 2012
Surimi	6.7–16.7	10,000	90	1.7–4	- Breaking force and deformation dependent on the electric field strength - Increase of protein aggregation - Higher level of residual SH groups - Inactivation was accelerated	Lu et al., 2015
Chymotrypsin inhibitor and trypsin inhibitor	11–22	50	0–100	3–15	- Higher protein incorporation efficiency - Higher film formation rate - Improved re-hydration capacity	Lei et al., 2007
Soy protein-lipid film	16.9	50	85	7–8	- Enhanced inactivation due to electrical effects - Enhanced inactivation due to electrical effects - No additional MEF effects	Castro et al., 2004
Enzymes						
Lipoxygenase	20–90	50	60 to 78	5	- Enhanced inactivation at shorter time processing and lower temperature when compared with conventional heating - It inactivates at lower processing time than conventional water blanching	Demirdöven & Baysal, 2014
Polyphenoloxidase			70 to 95		- Critical deactivation temperature decreases with the increase of the electric field strength	Icier et al., 2006
Pectinase			60 to 75	5–25		Icier et al., 2008
Alkaline phosphatase			55 to 70			Jakób et al., 2010
Beta-galactosidase			65 to 80		- Minor change in inactivation kinetic parameters - Minor change in inactivation kinetic parameters	
Pectinmethyl/esterase (PME)	42	50	770	0.6	- High destabilization - Higher rates of inactivation between 1 and 60 Hz - No significant effects	Samaranayake & Sastry, 2016
Peroxidase	20–50	50	30–100	0–10	- Activation at lower temperatures (< 70 °C)-Enhanced inactivation at higher temperatures	Samaranayake & Sastry, 2016
Peroxidase	20–40	50	60–90	5–25	(> 70 °C); Effect increased with electric field strength - Reduction activity by 98%	Leizerson & Shimomi, 2005
Pectin methyl/esterase	n.a.	50	54–66	2–120	- Reduction activity of 8% more than conventional treatment	Demirdöven & Baysal, 2014
Alkaline phosphatase			52–64	4–750	- Electric treatment (1 min) achieved similar inactivation as a conventional in 5 min	Makroo et al., 2017
Peroxidase			58–78	6–1500		
Pectin methyl/esterase	0.4	0 (DC) – 1000000	65	5		
Polygalacturonase	5 – 10.5	60	65–90	2.6–35	- Production of films with improved physical barrier properties (e.g. less permeable to water vapor) - Production of edible films with improved mechanical properties (e.g. increase of tensile strength and elongation-at-break of c.a. 9 and 18%, respectively)	Souza et al., 2009
Pectin methyl/esterase						
	n.a.	50	90–150	≈ 1–2		
	20–60	50	34–76	1.6		
	24	50	90	1		
Biopolymers						
Chitosan	50–200	50	60	-		
Chitosan	50–200	50	60	-		

(continued on next page)

Table 3 (continued)

Matrix	Processing conditions MEF/V/cm	Frequency/Hz	Temperature/°C	Time/min	Effects	Reference
Chitosan-Starch	7.6	25,000	70	30	- Chitosan films with more hydrophobic character - Starch films with more hydrophilic character - A useful tool for the incorporation of microcrystalline cellulose in films structure - Ability to change surface properties of polysaccharides	Coelho et al., 2017
<i>Microorganisms</i>						
<i>A. acidoterrestris</i> spores	30–50	50	70–90	0–30	- Higher lethality of spores, up to 5 log reduction	Baysal & Icier, 2010;
<i>E. coli</i> O157:H7, <i>S. Typhimurium</i> , <i>L. monocytogenes</i> , and MS-2 bacteriophage	12.1	60	25–90	0.17–1.7	- Synergetic bactericidal and virucidal effects with carvacrol	Kim et al., 2017
<i>Escherichia coli</i> O157:H7, <i>Salmonella enterica</i> serovar Typhimurium, and <i>Listeria monocytogenes</i>	30–60	20,000	55–60	0–0.5	- Additional bacterial inactivation at sublethal temperature - Cellular permeation increase	Park & Kang, 2013
<i>Escherichia coli</i> ATCC® 25922 and spores of <i>Bacillus licheniformis</i> ATCC® 14580	20–54	50	55–80		- Increased inactivation and D-time reduction	Pereira et al., 2007
<i>Bacillus coagulans</i> spores	13	60 and 10000	95–110	0–30	- Increased inactivation - Appreciable effects even at sublethal temperatures	Somavat et al., 2013
<i>S. cerevisiae</i>	10–20	60–60000	20–100	0–15	- Electroporation and leakage of cellular material	Yoon et al., 2002
<i>E. coli</i>	50–280	50	25	0–20	- Changes in cellular membrane - Cellular inactivation	Machado et al., 2010
<i>Lactobacillus acidophilus</i>	2	45–10,000	30	1200	- Cell permeabilization and lag phase reduction at low frequencies (60 Hz)	Loghavi et al., 2008
<i>Lactobacillus acidophilus</i>	15–40	60	30–40	Up to 3000	- Lag phase reduction up to 94%	Cho et al., 1996

on functional aspects of several molecules and living cells.

#### 4.1. Moderate electric fields in the biorefinery context

The conventional biochemical platform for biorefinery approach may involve several steps such as thermal processing, enzymatic hydrolysis and fermentation routes. Application of MEF and internal heat generation due OH effect allow to make faster thermal treatments, in a direct, volumetric and uniform way, avoiding issues related with limitations of heat conduction and convection commonly seen with indirect heating methods. OH has not a theoretical upper temperature boundary being well suited for thermal pre-treatments (i.e. microbial and enzyme inactivation) or thermal hydrolysis processes; high temperature can be attained in a short time processing without the use of external source of energy, being the only limitation the electrical conductivity of the sample to be treated. At sub-lethal temperatures the influence of MEF may also trigger different metabolic responses of enzymes which an important role in biotechnological processing applications, either by changing functional aspects (e.g. texture in cosmetics and foods), by catalyzing transformation of by-products or by hydrolyzing the matrix structure and facilitating the extraction of target biocompounds or fractions. The possibility of naturally combining electrical effects (i.e. moderate electric field and tunable electrical frequency) and ohmic heating treatments (Joule effect) to inactivate spoiling microorganisms and change functional properties of protein fractions, carbohydrates and enzymes in a very controlled and sustainable way are attracting attention showing potential to widening up the range of biotechnological applications in biorefinery far beyond the extraction of biocompounds.

##### 4.1.1. Biomass microbial stabilization

OH is seen as an alternative thermal method for pasteurization and sterilization being considered as one of the “emerging high-potential technologies for tomorrow” (De Vries et al., 2017). Among the several advantages of this electrical processing aforementioned, which include short heating times, uniform heating and less over processing (Jaeger et al., 2016; Vicente et al., 2014), stands out the possibility of enhancing thermal inactivation of microorganisms (including vegetative cells and spores), which has been extensively reviewed (Cappato et al., 2017; Jaeger et al., 2016; Vicente et al., 2014). It is consensual that thermal inactivation is always assured, but the majority of studies points out additional non-thermal effects in cell death (Baysal and Icier, 2010; Kim et al., 2017; Park and Kang, 2013; Somavat et al., 2013; Yoon et al., 2002). Enhanced microbial death kinetics through OH can be explained either by thermal permeabilization or by electroporation of cell membranes (formation of pores) resembling pulsed electric field treatments (Aghajanzadeh and Ziaifar, 2018; Jaeger et al., 2016). Electric effects contributing to inactivation can rely on the combined use of low electrical frequencies (50–60 Hz) and MEF intensity (< 1000 V/cm) even when applied at sub-lethal temperatures (Machado et al., 2010). Knowing that many bioresources, including the majority of agro-food by-products, seaweeds and microalgae, easily spoil due to their high moisture levels (Aspevik et al., 2017), the application of electro-heating technologies figures itself as a valid processing tool to be applied in the stabilization of biomass streams for further routes of biorefinery flow, such as fermentation (e.g. sugar-rich substrates).

##### 4.1.2. Fermentation

Application of MEF during fermentative processes were early reported by Cho et al. (1996), which observed a biological activation effect on growth *Lactobacillus acidophilus* under influence of alternating electric fields. This study reported that the lag period decreased by 94% under MEF in a fermentation at 30 °C when compared with conventional fermentation method. The possible explanation advanced was that MEF applied enhanced dislodgement of polar antimicrobials and other molecules adhered to cell walls and membranes, allowing to

minimize inhibitory action of fresh fermentation medium and thus improving absorption of nutrients. Strangely, no other relevant studies on the sub-lethal effect of MEF in microorganisms during fermentative processes could be found. More fundamental knowledge about influence of sub-lethal effects of MEF on the metabolic pathways of living cells should be encouraged, once can bring novel insights on the improvement of fermentation routes.

#### 4.1.3. Enzyme-assisted extraction

Contrary to microbial inactivation, information relating to non-thermal effects of MEF treatments on enzymes is still scarce (Samaranayake and Sastry, 2016b) and difficult to generalize. The majority of the results regarding enzymes thermal inactivation in the presence of different voltage gradients or electric fields suggest that electric or “non-thermal” effects are enzyme-dependent and may also vary in accordance with the matrix in which activity assays are being performed (Samaranayake and Sastry, 2016b; Vicente et al., 2014). Castro et al. (2004) they suggested that the presence of MEF may interfere with the metallic prosthetic groups present on lipoxigenase and polyphenoloxidase, causing enhanced inactivation. Jakóáb et al. (2010) based on thermodynamic analysis assumed additional inactivation effects of MEF may be linked with different distributions ions around enzyme molecules, thus affecting optimal enzymatic environment. Currently, among the several enzymes, the effects of MEF on catalytic reactions activated by pectin methyl-esterase (PME) are attracting attention in bioprocesses and food biotechnology. This enzyme cleaves the methyl ester bonds in pectin and is thus often used for clarification of fruit juices and softening of the cell walls of higher plants for the extraction of valuable fractions (Aghajanzadeh and Ziaifar, 2018; Samaranayake and Sastry, 2016a; Sharma et al., 2017). Much in the same way, OH treatment has been considered as an efficient alternative to the conventional method of PME inactivation in tomato juice (Makroo et al., 2017). Recently, Samaranayake and Sastry (2016b) showed that the application of electric fields of very low intensity (0.4 V/cm) at a temperature of 65 °C and electrical frequencies ranging from 1 to 60 Hz gives rise to enhanced inactivation of PME (up to 26%) in tomato homogenate. At higher electrical frequencies, this additional inactivation is not observed. It is suggested that low frequencies result in sufficient motion and displacement of enzyme molecules to increase the probability of additional collisions with water molecules from the same environment. The effects of increasing electric fields on PME activity were also assessed in tomato homogenate (Samaranayake and Sastry, 2016a) being shown that at higher temperatures (> 75 °C) the efficacy of the electric field in PME inactivation increases with increasing field strength up to 10.5 V/cm. Interestingly, a non-thermal activation effect in PME activity is reported to take place within MEF treatments at 70 °C (Samaranayake and Sastry, 2016a). Eventually, MEF may also increase reaction rates by promoting an increased accessibility of enzymes to the substrate or by enhancing electrophoretic motion.

It seems straightforward that enzyme inactivation can be increased when MEF is used, requiring treatments with less holding time and lower temperature than conventional heating methods due to the electrical effects, but enzyme activation may also happen. The influence of electrical variables on enzyme activity – i.e. electric field and frequency – still need more fundamental investigation but at a first instance MEF can offer an opportunity to e.g. regulate enzyme catalysis during bioprocesses. The combined application of enzymatic hydrolysis with assisted aqueous extraction using the both MEF and OH effects can be an interesting approach for the extraction of valued-added compounds.

## 4.2. Functionalization of extractable biopolymers

### 4.2.1. Proteins

Protein-rich wastes from animal and plant-derived origin are an

important and profusely available resource but still currently treated as by-product, being discarded or used for soil fertilization and animal feed purposes (Aspevik et al., 2017). Food waste materials rich in solids such as tomato and grape pomace may contain at least 10% (w/w) of extractable protein in their composition (Hegde et al., 2018). Protein-containing by-products from the meat industry (e.g. blood and collagen) are also attractive for the production of bioactive peptides (Ryder et al., 2016). These alternative proteins can be used in human nutrition due to their interesting biological and functional value, but generally they do not present the desirable technological and nutritional properties in their native form to be introduced in the food chain production. During the last decade, the impact of OH and the effects of MEF have been addressed on whey, soybean and fish muscle proteins, as well as in some biopolymers of carbohydrate origin with interesting functional features.

Whey from the production of cheese, and casein that in the past was seen as a by-product from the dairy industry is now recognized as “an outstanding example for a successfully processed by-product” (Raak et al., 2017) and thus an attractive starting material for the development of protein-based food ingredients or functional systems (Ramos et al., 2017; You et al., 2017). Within this context, electro-heating processing was successfully used to tailor the denaturation of whey proteins and change the morphology of the produced protein aggregates (Pereira et al., 2016b). This was achieved by turning on OH as fast as possible, thus reducing the heating charge to achieve a target temperature, in combination with an increase of the electric field applied during heating. These electrical-heating treatments favored the appearance of linear or fibril-like protein aggregates that can act as important structuring agents for the development of protein gel networks which may further be used in the encapsulation of drugs, bioactive compounds or nutrients. For example, MEF can allow incorporating significant amounts of iron (about 33 mmol L<sup>-1</sup> of ferrous sulfate) within a whey protein-based hydrogel without affecting negatively the stability of the microstructure of the protein system (Pereira et al., 2017). Electric fields can also be applied to transform whey emulsions into gelled protein systems with distinctive properties. It was pointed out that small structural changes at the nanoscale level imposed by MEF should not be overlooked, once they can impact the macrostructural properties of gels made from whey globular proteins, such as lactoferrin (de Figueiredo Furtado et al.).

Myofibrillar proteins from meat muscles present unique gelling properties making them one of the most widely used functional ingredients in the development of hydrogel systems. During fast OH the inactivation of proteolytic enzymes rapidly occurs and renders more myofibrillar protein available to form disulfide bonds, thus stabilizing gel networks (Tadpitchayangkoon et al., 2012). These authors also point out that some of these mechanisms need to be better elucidated, once protein conformational changes, as well as different patterns of protein denaturation and aggregation induced by MEF may be playing a role on development of distinctive protein gel networks. Recently, Lu et al. (2015) that OH treatments can be used to accelerate trypsin and chymotrypsin inhibitors inactivation to levels of 13% and 53%, respectively and enhance the formation of protein aggregates, due to either by thermal, electrical (MEF) or electrochemical effects which can change the balance and interchange between protein covalent bonds (SS, disulfide-disulfide) and free thiol groups (SH, sulfhydryl).

### 4.2.2. Carbohydrates

Biopolymers of carbohydrate origin, such as chitosan, cellulose, pectin and starch (among others), can be recovered from a variety of agricultural commodities and wastes and be valued into new products, such as edible thin films and coatings to protect fresh or processed foods as a way to extend their shelf-life (Baron et al., 2017). Currently, some of these materials, such as chitosan, are also playing an important role on several applications in the biomedical field and tissue engineering (e.g. development of scaffolds) due to their biodegradable, non-toxic

and non-allergenic, and thus biocompatible nature (Ahmed and Ikram, 2016). Souza et al. (2009, 2010) studied the effects of MEF treatments on transport and mechanical properties of chitosan coatings and films structure, concluding that an increase in the permeability to water vapor, oxygen and carbon dioxide, as well as a decrease in surface roughness can be achieved over conventional methods of production. More recently, MEF treatments were successfully used to tailor properties of starch and chitosan films reinforced with microcrystalline cellulose (Coelho et al., 2017). Results showed that application of MEF in chitosan lead to more hydrophobic films, with lower water vapor permeability values and higher tensile strength, corroborating previous works (Souza et al., 2009; Souza et al., 2010), while MEF-treated starch films presented a more hydrophilic character and lower tensile strength, when both were compared with films obtained from conventional methods of production. Overall, the reviewed electrical treatments seem to have the potential to modify the surface of these polysaccharide-based films, thus bringing new insights regarding changes of their biofunctionality and bulk properties that are worthy of more fundamental investigation.

#### 4.2.3. Lipids

Fats and oils are not able to conduct electricity, working instead as electrical insulators. This limits the application of electro-heating processing to this kind of products, and justifies the scarcity of information available about electrical effects on lipids. Nevertheless, OH has been used to produce protein-lipid films from soybean milk, with claimed advantages on rehydration capacity and film formation rate (Lei et al., 2007). Electric fields applied during OH seem to improve, or at least not affect negatively, fat stability of products prone to enzymatic or physical liberation of free fatty acids (Pereira et al., 2008; Nandi et al., 2017).

### 5. Processing and energetic aspects

Novel and emerging technologies not only have to prove effective and advantageous at the processing level; they have to be technically feasible, sustainable and economically competitive. The major impairments associated with these technologies are always their implementation, as the associated costs tend to be higher than those of the conventional technologies and there is a lack of operational information and associated costs (Elsayed and Mohammed, 2016; Pereira and Vicente, 2010). In order to assess the profitability of a new technology, several aspects have to be taken into account, such as investment in equipment, energetic costs, and operational aspects such as efficiency gains and reduction or elimination of process requirements (e.g. utilization of solvents or mechanical methods to increase extraction).

#### 5.1. Processing demands and scaling

In principle, electrotechnologies are almost linearly scalable and involve low maintenance costs, since their application does not involve diffusional processes, mechanical stress or moving parts (Pereira and Vicente, 2010). However, their industrialization offers some challenges, as large volumes processing implies larger treatment chambers or flow rates, larger electrodes surface areas and electrode gaps. Consequently, the demands on the generators increase, implying high energetic capacity, increase of output voltage and higher frequency of pulses (Kotmik et al., 2015). Obtaining power sources capable of high voltage and current output with defined pulse/wave shape and frequency is still a challenge (Golberg et al., 2016). Nonetheless, developments in generators and control systems, along with increasing application of MEF and PEF systems in the food industry, are contributing for cost reduction and increase of reliability of these systems. In addition, optimization of several factors such as materials used, design of electric treatment chambers and continuous operation mode are pushing these processes towards industrial feasibility (Golberg et al., 2016; Mahnic

and Miklavc, 2014). In this scenario, HVED is still the less developed technology, because not only it is the most recent, but also it is the more demanding in terms of generator specifications and challenging in terms of materials used and chamber design (Vorobiev and Lebovka, 2013).

#### 5.2. Energy consumption and environmental impact

Electrotechnologies presented here are considered environmentally friendly once they may eliminate, or at least diminish, the use of water and thus production of wastewaters (e.g. avoiding the use of steam systems and boilers), and may use a renewable source of energy (e.g. hydroelectric power) to produce electricity. Furthermore, in general, electrical processing needs lower energy consumption compared with conventional pre-treatment methods and extraction technologies.

The energy input associated with PEF treatment varies with the material to be treated; commonly it ranges from 1 to 15 kJ/kg in soft tissues such as pulps and peels. In contrast, conventional treatments (such as mechanical or enzymatic processing) require from 20 to 100 kJ/kg to achieve similar results (Golberg et al., 2016; Puértolas and Barba, 2016; Vorobiev and Lebovka, 2010). In hard and resistant materials such as seeds, PEF energy requirements rise to 100–800 kJ/kg, placing it on the energetic range of HVED treatments (Boussetta and Vorobiev, 2014). For HVED, limited data are available and few industrial or economic studies were performed. However it seems that despite having similar energetic levels to PEF, HVED is more advantageous for the processing of resistant particulate material as its more efficient in promoting extraction on these matrices (Liu et al., 2011; Parniakov et al., 2014).

MEF treatments often involve OH as a thermal process and the energy requirements will be dependent of the materials heat capacity and thermal elevation needed. OH energetic efficiency is above 90% and compared with the less efficient conventional thermal processing it can achieve energy savings up to 70% (Pereira and Vicente, 2010; Varghese et al., 2012). When applied to extraction processes, OH has shown energy inputs between 30 and 180 kJ/kg (Pereira et al., 2016a), being significantly higher than PEF and at similar levels of mechanical and enzymatic processing. However, as a thermal process, OH also achieves microbial and enzymatic inactivation, but in this case at significant lower energy inputs than PEF would require (i.e. 40–1000 kJ/kg) (Vorobiev and Lebovka, 2013).

Higher energetic efficiency will inevitably result in the reduction of overall energetic consumption. Along with the increase of bioprocessing and recovery efficiency, and in some cases reduction or elimination of solvent use, electrotechnologies are likely to significantly contribute to reduce the use of non-renewable resources, increase the added value of wastes and by-products and to the development of a green, sustainable and circular economy.

### 6. Future challenges

Innovative processing tools are needed to manage and transform, in a sustainable and profitable fashion, endogenous bioresources and unavoidable local wastes into new products and value-added compounds (e.g. biopolymers, pigments, bioactive peptides, among others), in a bioeconomical perspective. This is a huge challenge, but an essential one in terms of economic and environmental benefits, as well as of public health (Williams et al., 2015). In order to support an emergent bioeconomy and convey underrated bioresources to the food supply chain or to other value-added markets (such as cosmetics and pharmaceuticals), electrotechnologies need also to be economically feasible and competitive. The core of the successful approach of electrotechnologies processing in valorization of bioresources will be anchored in the following points: 1) a more fundamental understanding about the effects of electricity on biological cells, biomacromolecules and biopolymers; 2) a clear differentiation between thermal, electrical and

electrochemical effects promoted by electrotechnologies, once this knowledge will allow controlling and designing specific and well-oriented treatments; 3) “back to basics”, meaning that fundamental and ancestral sustainable ways of processing food (e.g. fermentation and enzymatic catalysis) could be strategically combined with innovative processing seeking sustainability and synergistic effects; 4) “less is more”, applied research should be restricted to strong evidences that valorization will sensibly be cost- and resource-effective when scaled-up; and 5) a strategy of reverse engineering should be useful for preliminary screening of nutritional and molecular composition of heterogeneous food by-products to be valorized, as well as to enhance knowledge of physical and chemical changes that may occur under processing using electrotechnologies.

## 7. Conclusions

Valorization of bioresources encompasses a multidisciplinary approach that can cover several technological operations from the processing point of view, linked with preservation, extraction and transformation. The so-called novel and emergent electrotechnologies fit in the concept of “Green and Sustainable Processing”, assuring environmental benefits through increasing the overall energy efficiency, water saving, reduced emissions and reduced use of non-renewable energy sources. In particular, MEF can be used both as extraction tool and as functionalization agent of biological compounds broadening their application range. The substantial increase of MEF-based plants installed worldwide suggests that its application for waste recovery will be straightforward.

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