Pulsed Electrical Technologies Assisted Polyphenols Extraction from Agricultural Plants and Bioresources: A Review

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Abstract: The application of pulsed electrical technologies such as high voltage electrical discharges (HVED) and pulsed electric fields (PEF) have been currently proposed for promoting biocompounds extraction. Even if their principles of action are different, both of these techniques have shown to be efficient for the enhancement of polyphenols extraction from different raw materials as compared to control extraction. Depending on the product, the energy consumption, the cell disruption, the polyphenols composition, the extraction and purification steps are different when applying PEF or HVED. This paper thus reviews the current status of research on the application of HVED and PEF for extraction and purification of polyphenols from plants.

Keywords: Polyphenols, Extraction, Selectivity, Purification, Pulsed electric field, High voltage electrical discharges.

1. INTRODUCTION

Nowadays, the interest for natural ingredients has been growing due to their beneficial health effects. Numerous epidemiological studies have demonstrated the protective effect of polyphenols in fruits and vegetables against degenerative diseases because of their antioxidant activity. For example, studies have shown that polyphenols have anti-viral, anti-inflammatory, anti-tumor and had a beneficial role in the prevention of cancer and cardiovascular diseases [1, 2]. Polyphenols or phenolic compounds are specific plant secondary metabolites. The structural element is a benzene ring with one or more hydroxyl, free or engaged with a substituent (alkyl, ester, sugar) [3] groups. The molecular weight of the phenolic compounds range from simple compounds (<100 g/mol) to highly polymerized structures (> 30 000 g/mol).

The conventional method of polyphenols recovery from plant is based on a solid-liquid solvent extraction. Depending on the type of solvent used, the cell membranes could be more vulnerable which facilitates the release of polyphenols. Many methods have been developed to intensify the extraction process such as microwave [4], high-pressure [5], supercritical fluid extraction [6] and ultrasound [7]. In particular, the pulsed electrical technologies (high voltage electrical discharges (HVED) and pulsed electric fields (PEF)) are techniques which act on the membranes and / or cell walls, thus facilitating the extraction of biocomponents.

Initially designed for military or scientific applications of very high energy, HVED can now be adapted for civil applications. They can be classified into three categories: (1) applications in the field of lasers, X-rays and microwaves (mega-joule laser, synchrotron sun, radars, jammers ...) (2) applications for specific test means (lightning tests, electric launchers ...), (3) civil applications processing gases (NOx, SOx, dust smoke ...), liquid handling (removing bacteria, pasteurization, cold extraction of cellular compounds [8-9], flocculation sludge ...), treatment of solid (waste separation and crushing, grinding products, peeling concrete, ceramic sintering ...). This review will focus on the use of this technology for the extraction of polyphenols.

The use of pulsed electric fields in food industry began in the 1960s [10]. In the 1990s, new pilot equipments of pulsed electric fields have been developed. In Germany, the Elsteril process allowed treating various liquid food while the Elcrack process was designed in order to extract fat from fish. Commercial food products treated by PEF appeared in the United States by using the PurePulse system developed by Maxwell Laboratories in 1993 [11]. More recently (2001 – 2005), PEF have been applied on vine grapes at the industrial scale with the KEA-Wein system [12] (Germany) in order to increase the content of polyphenols in wine. The two main applications of PEF are thus the microbial reduction of food and the extraction of intracellular compounds from plant cells [13-14].

This review will focus and compare the potential of these two-pulsed electrotechnologies (PEF and HVED) for the enhancement of polyphenols extraction from

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E-ISSN: 2408-9826/15

plant. Various criteria will be discussed in order to help choosing the appropriate technology regarding the initial raw material and the overall process requirements.

2. PRINCIPLES OF PULSED ELECTROTECHNOLOGIES

Action Mechanism of PEF

The application of an external pulsed electrical field can induce the formation of pore on the cell membrane: this phenomenon is called electroporation [10]. The capacitor model has been proposed to explain the PEF mechanism. The cell membrane which is composed of two lipidic layers can be represented by a capacitor *c*. The cell cytoplasm acts as an electrical conductor. The conductivity of the extracellular media is represented by a resistor in parallel *r* and two resistances in series.

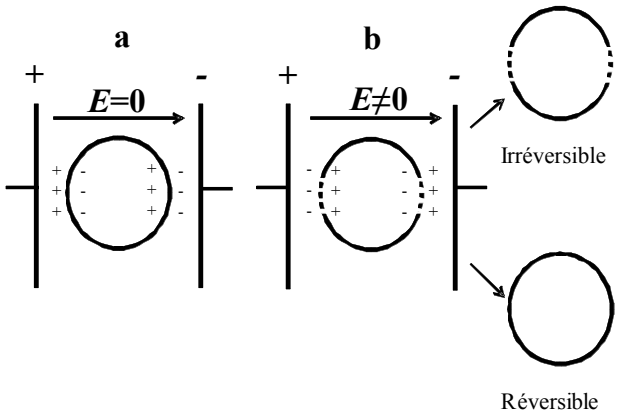

Figure 1: Membrane electroporation by PEF (a: cell before permeabilisation, b:cell after permeabilisation).

When an electric field is applied *E*, there is charges accumulation on both sides of the membrane. The cell membrane is polarized and a transmembrane potential appears V_m. For V_m values higher than the dielectric characteristic of the membrane (≈ 1 V) [15], pores are formed on the membrane. This phenomenon would be similar to the breakdown of an electrical capacitor. Note that the pore formed can be either reversible or irreversible depending on the applied electric field intensity (Figure **1**).

The PEF effect has also been observed inside the cell. The photographs obtained by transmission electronic microscopy show the effect of PEF on yeast cells (*S. cerevisiae*) samples (Figure **2**). For PEF treated yeasts (20 kV/cm), the detachment of the cell membrane to the wall is observed as well as a modification of the intracellular content organization [16]. PEF seem to be also responsible for the leakage of intracellular compounds between the detached membrane and the cell wall.

Action Mechanism of HVED

The HVED expression include several phenomenon that can appear separately or in combination: (1) the "predischarge" phenomenon indicating that the applied electric field plays the main role, (2) the "corona" phenomenon indicating that the ionization phenomenon predominates with the formation of an electron avalanche (streamers), (3) the "arc" phenomenon which refers to the formation of shock waves (Figure **3**), (4) the "leader" phenomenon indicating that thermalization plays a significant role, (5) the "electron beam" (non-thermal plasmas) in which electrons are very energetic. Note that plasma is a gas whose molecules are ionized. HVED can induce thermal, photonic, acoustic and/or mechanical effects. HVED

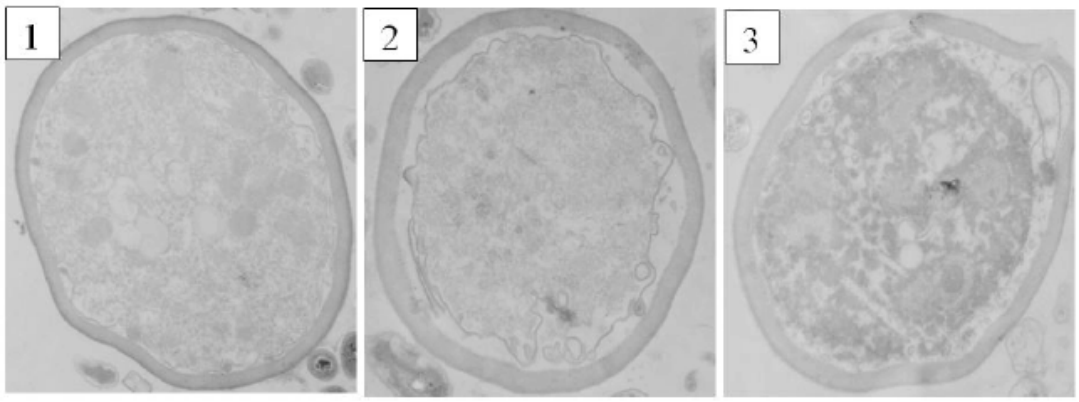

Figure 2: Photographs (transmission electronic microscopy) of untreated and PEF treated yeasts (S. cerevisiae). 1-untreated cells, 2- PEF treated cells (20 kV/cm, 120 pulses of 1 µs), 3-PEF treated cells (20 kV/cm, 160 pulses of 1µs) (X 11 500) [16].

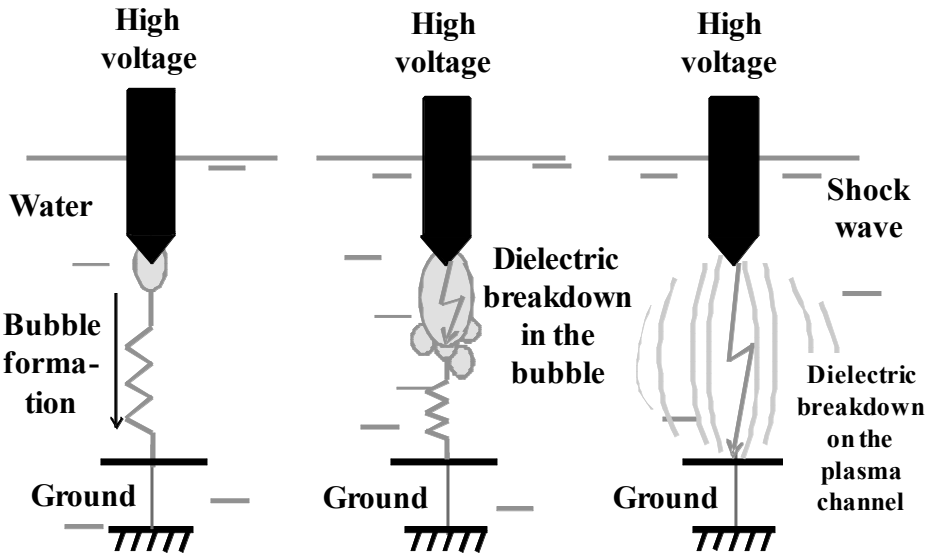

Figure 3: Main and secondary phenomena induced by HVED [17].

can produce free radicals that make very reactive environments.

The phenomena involved during the formation of HVED strongly depend on the environment in which they are applied: aqueous or gaseous medium. Only HVED applied in liquids will be discussed here. Despite many studies carried out on electrical discharges in liquids, there was no unanimity on the interpretation of phenomenology. Regarding the case of water, all authors agree that there is at least two types of discharges in liquids: slow discharge (subsonic) and rapid discharge (supersonic).

In the case of subsonic discharge (Figure **4**), the discharge propagates through the gas bubbles by using a thermal process [17]. The energy injected into the liquid at the capacitor discharge moment allows thermalizing the environment. The use of electrodes with point-plane geometry favors the concentration of field lines in the vicinity of the point; this zone is thus preferably heated. A bubble of gas, presumably of water vapor, thus appears in the vicinity of the point where there is a relatively high electric field (~ 80 kV / cm). Note that to create a dielectric breakdown in the bubble, the field value is more than two times lower (30 kV/cm in air at 27 °C) [18]. Ultraviolet rays and infrared light appear as a result of the breakdown of a bubble [17]. Therefore, the temperatures reached are high enough to thermalize inside the bubble and near the bubble that undergoes breakdown and create new bubbles. The arc channel being conductive, the potential is dropped on the tip end of the bubble and the phenomenon can thus propagate. The volume of bubble then tends to fill the inter-electrode space. The complete dielectric breakdown of the medium occurs

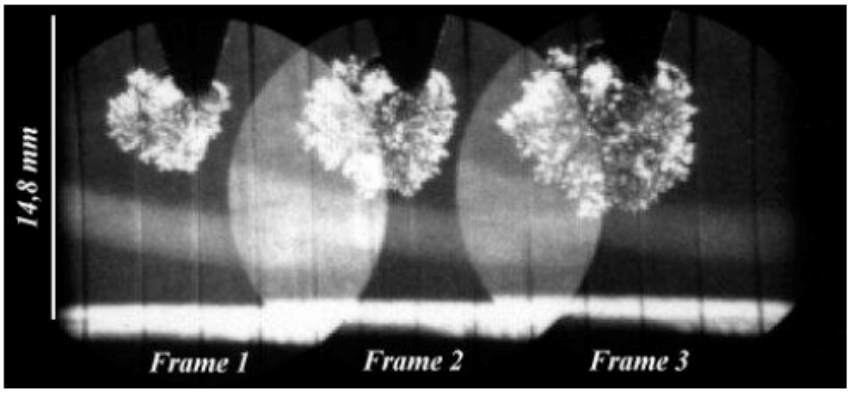

Figure 4: Photograph of a subsonic discharge in water (before the dielectric breakdown) [17].

when the gas bubbles reach the electrode plate. An arc is created between the electrodes. The expansion of the arc channel leads to the pressure wave generation (also called shock wave) that propagates into the liquid product to be treated [19]. When the shock wave comes into contact with the cell membranes of the product, the latter is damaged. The intracellular content is released to the surroundings.

For supersonic discharge (Figure **5**), the discharge propagates through filamentous channels. Given the rapidity of events, the phases of initiation and development of supersonic discharges in water are much more difficult to analyze. Some authors state that supersonic discharge would develop in a gaseous medium after a phase change by vaporization and thus they apply the theory of the discharges in the gas to describe the phenomena. Others reject the thesis that an electronic avalanche could be developed in water [20]. The work of Gavrilov *et al*. (1994), Kukhta *et al*. (1996), and Kukhta al. (1999) [26-28] highlight supersonic leaders (presence of thermalization) whose development is accompanied by a pressure wave.

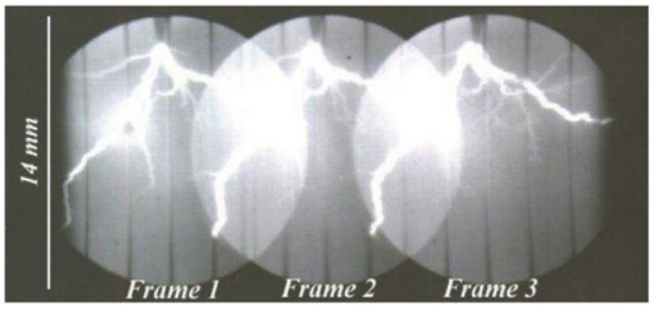

Figure 5: Photograph of a supersonic discharge in water (before the dielectric breakdown)[17].

In addition to UV radiation, infrared radiation and shock waves generation, an electric discharge in water produces chemically active species (radicals $H^{\bullet}$, $HO^{\bullet}$, $O^{\bullet}$, $O_2^{\bullet -}$, and the peroxide hydrogen H_2O_2), highly energetic electrons and ozone (O_3) [24]. These chemical species are known for their role in the degradation of organic compounds by oxidation. The generation of these active species by the discharge is closely related to the conductivity of the solution, the applied voltage and the geometry of the electrodes.

3. ROLE OF PEF AND HVED ON CELL AND TISSUE DISRUPTION

The cell damage induced by electrical treatment can be indirectly quantified by measuring the electrical conductivity of a solution. This measure allows the determination of the charged particle concentration. It can be used to follow the kinetics of extraction of the total solute [25]. The cell permeabilization index (or cell denaturation) Z [13] can be determined by the following equation:

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i} \quad (1)$$

Where σ is the electrical conductivity at time t (S/m); σ_i, the electrical conductivity of the intact (non damaged) product (S/m) ; σ_d, the electrical conductivity of the completely damaged product (S/m). The application of this equation gives Z = 0 for an intact tissue and Z = 1 for a completely damaged tissue.

The effect of HVED and PEF on the *cell denaturation Z* was studied on various raw materials. For example, this index was determined for different treatment times (1 – 7 ms) of PEF or HVED from sesame cake [26]. It has been shown that the cell damage was increasing as a function of the treatment time for both PEF and HVED. However, this enhance went up to a certain critical treatment time. After 3 ms, the PEF treated samples reached a maximum of 67 % cell damage. For HVED, the maximal degradation index (90 %) was reached after applying only 2 ms. This suggests that after these values most of the cell membranes were permeabilised and most of the cell walls were disrupted (in the case of discharge). In the case of vineshoot, PEF and HVED also induce cell damage and the damage degree Z increased with higher treatment time. HVED leads to higher cell damage than PEF [27]. For instance, 20% of cells were damaged after 5 ms with PEF and only 0.5 ms with HVED.

The *nature of raw materials,* and in particular, the tissue structure of the product, has an effect on the denaturation index. In the case of HVED, the maximum cell damage (100 %) was reached after 5 ms of treatment of flaxseed cake [34]. With grape pomace, the maximal cell damage was obtained after only 0.8 ms of HVED [29, 30]. When applying PEF on grape skins, the maximum cell damage (100 %) was attained after 1 s treatment [31]. Different optimal treatment times for a maximal disintegration index were found for other products such as sugar-beets [32, 33], potatoes [34, 36], apple [37]; grape [38]; chicory [39, 40]. An optimization study for each product is thus required. In general HVED was more efficient that PEF and both treatments showed higher yield when compared to control. Indeed, different phenomena are involved in

each methodology. PEF treatment causes damage to the cell membranes and HVED damages the cell membranes and cell walls. The arc which is formed inside the treatment chamber with HVED, increases significantly the cell damage, as showed by Boussetta *et al*. (2013) [41]. Moreover, the arching effects in the HVED treatments have as consequence the grinding of the cake, since it produces shock waves that cause a highly turbulent mixing environment, improving cell disruption [42].

The cell disintegration by PEF or HVED allows the release of intracellular compounds thus enhancing the ***extraction process***. In particular, a correlation has been found between the denaturation index and the content of extracted polyphenols for sesame cake [26], flaxseed cake [28] and for vineshoots [27]. However, this relationship is not linear. The extraction of polyphenols doesn't necessarily starts when the first cells are disrupted, since a minimal damage per cell or number of damaged cells is required to enhance the biomolecules extraction. With vineshoot, a miminum treatment time of 0.4 ms and 0.9 ms was required to observe effective polyphenols extraction by HVED and PEF respectively [27]. Therefore, a threshold of cellular damage should be determined for each pretreatment, above which the enhancement of polyphenols extractions becomes significant.

At a macroscopic scale, it has been seen that PEF and HVED have different effect on the ***tissue structure***. Vine shoots had an intact aspect after being subjected to PEF. On the contrary, the fragmentation of vine shoots was clearly visible with HVED. The same observations were done for grape seeds, sesame cake, and grape pomace. The cavitation phenomena and shock waves induced by HVED seem to be the cause of the product fragmentation [41].

4. ENERGY CONSUMPTION REQUIREMENTS FOR EFFECTIVE EXTRACTION

When applying the electrical treatments (PEF or HVED) for the enhancement of the extraction of polyphenols from various raw materials, the main operating parameter is the treatment input energy. Polyphenols are quite polar compounds. Their extraction is thus enhanced in the presence of alcoholic solvents. Although there is no solvent able to extract all groups of polyphenols, ethanol is often used as a co-solvent. This green solvent is also widely accepted as a safe solvent. On the other hand, when the solid to liquid diffusion is carried out in hydro-alcoholic solvent, the extraction becomes more selective thus reducing/limiting the presence of some proteins and sugars in the final extract.

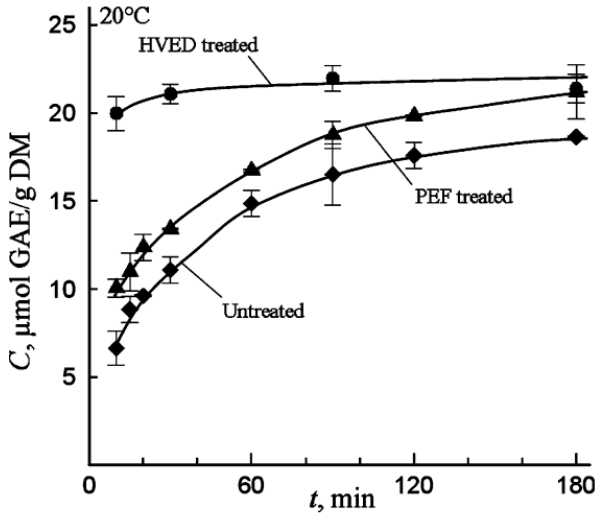

Figure 6: Content of total polyphenols C versus extraction time t for untreated and PEF-treated grape skins at 20°C (PEF treatment : E=1300 V/cm, t_t=1 s; HVED treatment: U=40 kV, t_t=120 s) [31].

For example, in the case of grape skins (Figure **6**), Boussetta *et al*., (2009) [31] have shown that both PEF and HVED had a positive effect on the extraction of polyphenols and total solutes. The amount of polyphenol extracts was significantly higher immediately after HVED (40 kJ/kg) (a four times increase as compared to a control extraction) and then reached a maximum. After application of PEF (1300 V/cm, 200 kJ/kg), the polyphenol content was increased twice. The initial extraction rates are different for control extraction and PEF or HVED assisted extraction but the final amounts of polyphenols are the same after 3 h of extraction. When varying the treatment input energy, ***optimum conditions*** have been determined. For example, the energy input of HVED has been varied for the treatment of aqueous suspension of grape pomace [30]. The rate of extracted polyphenols was initially increasing and then decreasing. There was an optimum extraction of polyphenols at 80 kJ/kg. The maximum extraction rate of total polyphenols was 1.37 ± 0.11 g g GAE/100 DM with a corresponding antioxidant activity of 23.02 ± 3.06 g TEAC / kg DM. The same trend was observed for individual phenolic compounds (catechin, epicatechin, quercetin-3-O-glucoside and kaempferol-3-O-glucoside). Beyond that optimal energy, the formation of free radicals and ozone during HVED seemed to be responsible for the degradation of the extracted polyphenols. Similar trends were obtained at

the ***pilot scale*** [42]. An optimal input energy of HVED treatment has been determined for the extraction of polyphenols from grape pomace: 100-160 kJ/kg (laboratory tests) and 400 kJ/kg (pilot testing). In both cases, HVED intensified the extraction of polyphenols by a factor of 6-7 as compared to a control extraction (diffusion without treatment).

On the other hand, the *nature of the raw materials* has a significant effect on the optimal treatment input energy for both PEF and HVED. When PEF is applied on dried product, the electrical operating parameters have to be adjusted. For example, the optimal processing conditions for polyphenols extraction from dried grape seeds are a PEF treatment at 384 kJ/kg with high electric field strength of 20 kV/cm, a treatment temperature of 50 °C and an extraction solvent containing 30 % ethanol [16]. Note that for this dried product, the experimental conditions are rather severe: there is a need of combining high electric field/input energy and moderate treatment temperature for an effective extraction. The content of extracted polyphenols was about 7 g GAE/100 DM. After pretreatment in these optimum conditions, the liquid to solid extraction performed at 50 °C for 60 minutes allowed reaching the maximum polyphenols content of 9 g GAE/100 DM. This rate of polyphenols was reached four times faster after HVED-assisted diffusion (40 kV, 64 kJ/kg) and from grinding assisted diffusion. The type of product has also an effect on the optimal HVED treatment input energy. For example, grape stems, that are rich in lignin, seem to be more resistant to HVED as the highest levels of polyphenols were obtained with higher values of energy (400 kJ/kg at the pilot scale, 213 kJ/kg at the laboratory scale). On the contrary, the grape skins appear to be more sensitive to HVED; energy of 133 kJ / kg was sufficient at the pilot scale to extract the maximum polyphenols content. A similar amount of polyphenols was obtained at the laboratory scale after a treatment at only 53 kJ / kg. Another product (vine shoot) which is rich in lignin but also dried required a minimum of input energy of 254 kJ/kg and 762 kJ/kg for effective polyphenols extraction by HVED and PEF respectively [27]. When treating oilseed residues of low water content, the input energy was also rather high for both HVED and PEF ranging from 168 kJ/kg to 300 kJ/kg from linseed cake [34] and linseed hulls [43].

5. IMPACT ON POLYPHENOLS QUALITY AND FUNCTIONALITY

The effect of electrical technologies on the *polyphenols composition* as compared to control extraction (without treatment) has been checked in several studies. It has been shown that the different groups of extracted polyphenols are not modified by the application of the treatment of PEF or HVED. From grape pomace, the same main polyphenols have been identified in both treated and untreated samples: catechin, epicatechin, quercetin-*3*-*O*-glucoside and kaempferol-*3*-*O*-glucoside and oenin [30]. All these compounds are typical of Chardonnay grape pomace and were also found in untreated samples. The same polyphenols composition of treated (PEF or HVED) and untreated extracts was also found from vineshoot samples: epicatechin, resveratrol and kaempferol [27]. In all cases and in concordance with the results of total polyphenols (usually measured by the Folin-ciocalteu method), the quantity of the different polyphenol compounds is always higher from treated samples than from untreated ones. In particular, HVED results in higher polyphenols compounds rates as compared to PEF when the same treatment input energy is applied.

However, the proportion of the different polyphenols compounds is not always the same with treated samples as compared to untreated ones. The application of PEF or HVED has an effect on *the extraction selectivity*.

The polyphenols composition of extracts (Figure **7**) obtained from grape skins at 20 °C after 60 min of PEF or HVED assisted extraction was determined by HPLC [31]. The HPLC profiles of these extracts were quite similar. Four main components were identified by comparing their UV-visible spectrum, the retention times and the mass spectra with the reference compounds. Flavanols (catechin and epicatechin) and flavonols (quercetin-3-O-glucoside and kaempferol-3-O-glucoside) were identified. However, the HVED assisted extraction allowed extracting more catechin and epicatechin than the PEF assisted extraction or the control experiment. This difference was attributed to the tissue fragmentation caused by HVED while PEF did not affect the tissue structure.

The effect of PEF and HVED on *polyphenols functionality* was also investigated. Polyphenols are known to be interesting antioxidant biomolecules which can prevent from several diseases. The antioxidant activity of extracts from treated samples has been thus evaluated. The antioxidant activity of PEF and HVED treated samples was higher than that of the control [16] which means that the polyphenols still remain active after the electrically assisted extraction. Depending on the extraction solvent, the antioxidant activity of

extracts could still be increased. When applying HVED on grape pomace and performing the solid to liquid extraction with 30% of ethanol in water, a maximal polyphenols content (2.8±0.4 g GAE/100 g DM) was obtained which was three times higher than that obtained with pure water. The corresponding antioxidant activity was also the highest in these conditions (66.8±3.1 g TEAC/kg MS).

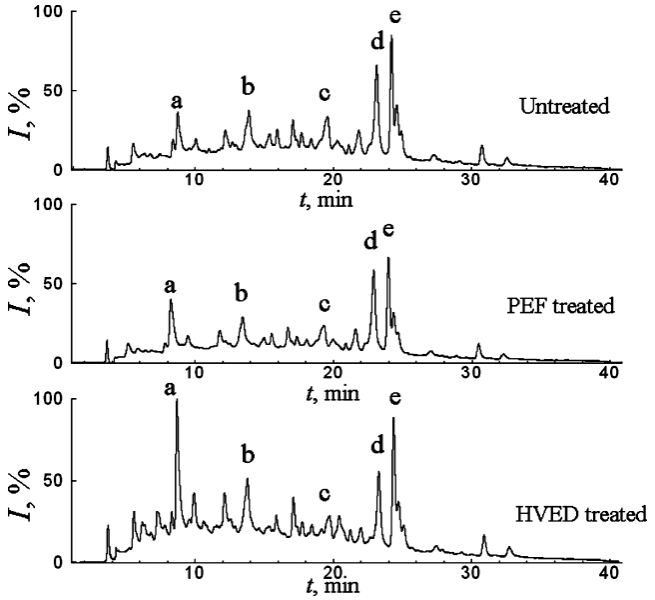

Figure 7: HPLC profiles from the extracts obtained at 20°C after 60 min of extraction for untreated, PEF-treated and HVED-treated grape skins. Identified compounds are catechin (a), epicatechin (b), quercetin-3-O-glucoside (c), kaempferol-3-O-glucoside (d). (PEF treatment : E=1300 V/cm, t_i=1 s; HVED treatment: U=40 kV, t_i=120 s) [31].

6. EFFECT ON SUBSEQUENT EXTRACTS PURIFICATION

The purification of extracts aimed at removing the residual solid particles but also the undesirable molecules such as proteins and sugars.

Even if PEF has been shown to be less effective for polyphenols extraction as compared to HVED, it has the advantage of preserving the structure of the product which will have consequent effect on the subsequent extracts purification. PEF act by electroporation of cell membranes but do not fragment the product. On the contrary, HVED damage both cell walls and cell membranes. The shock waves and the cavitation bubbles produced during treatment can alter and disrupt the product thus resulting in the fragmentation of the product according to the treatment energy input. This mechanical effect is similar to the grinding which can reduce the product into fine particles. For example, the diameters of untreated grape seeds and PEF treated samples were similar (about 4,000 mm) [16]. However, fine particles (dust on the surface of seeds) of about 10-20 µm were also detected in the suspension after simple diffusion and diffusion assisted by PEF. HVED reduced by 20 times the seed size (about 200 µm in diameter). The grinding also decreased the seed size (about 400 µm in diameter). The *centrifugation* is the first step required after the solid to liquid extraction in order to separate the solids residues from the extracts rich in polyphenols. The measure of the light transmission through the samples during centrifugation informs about the difficulty of the solid to liquid separation. Results have shown that the solid liquid separation was faster for suspensions treated with PEF than those treated by HVED and from crushed seeds. The presence of fine particles makes longer the centrifugation separation.

The two main purification processes of polyphenols extracts are the membrane filtration and the use of adsorbents.

Membrane technologies such as ultrafiltration and microfiltration have been widely used for the subsequent step of polyphenol purification and concentration [44-45]. Loginov *et al*. (2013) [46] used ultrafiltration process to purify polyphenol flaxseed hull extracts by separating them from proteins. Liu *et al*. (2011) [47] used dead-end ultrafiltration to concentrate polyphenol extracts resulting from HVED-treated grape pomace. The fouling process decreases filtration flux and affects filtrate quality by modifying membrane permeability and molecular selectivity [48]. The effect of PEF and HVED on the filtration efficiency has also been studied. Ultrafiltration of vine shoots extracts on polyethersulfone (PES) membranes with a molecular weight cut-off of 50 kDa was shown to concentrate polyphenols in the retentates [56]. Dead-end ultrafiltration without stirring was conducted to compare the cake specific resistance and the membrane resistance for the control and the PEF or HVED treated extracts. In general, the filterability of PEF extracts was easier than that of the HVED extracts. The specific cake resistance was the highest for HVED (3.8×10^{13} m/kg), followed by PEF (1.9×10^{13} m/kg), and then control (0.55×10^{13} m/kg). The same tendency was observed for the membrane resistance. A relationship was found between the disintegration index (Z) and the ultrafiltration parameters (membrane and cake resistances). The higher the pretreatment-induced cellular damages are, the greater the specific cake

resistances are. However, an increased cellular damage also results on a massive polyphenol extraction from vine shoots. A correlation was therefore observed between polyphenol concentration and membrane fouling phenomenon: HVED allowed the extraction of higher amount of polyphenols as compared to PEF but the filterability of the HVED extracts was also the worst: the filtrate flux was lower. The polyphenols retention and concentration were however higher with HVED indicating the possible role of polyphenols in membrane fouling during ultrafiltration of these extracts.

Purification of extracts can be also performed by using *adsorbents*. For example, the solid phase extraction can retain the polyphenols initially present in the liquid phase (sample) onto a solid phase (adsorbent). This technique is based on a chromatographic process; the solid support acts as a stationary phase, the solvent of the sample and then the elution solvent successively play the role of mobile phase. For example, silica has been used for the purification of extracts from grape pomace, grape skins, grape stems and grape seeds [16]. For most of these products, the adsorption rate of polyphenols on the resin (> 93%) and the overall purification yields (up to 87%) were relatively high except for the seeds. About 60% of proteins and 74% of sugars were removed by this technique. On the other hand, when the extracts were obtained from PEF or HVED assisted diffusion, polyphenols appear to have a lower affinity with the adsorbent phase (adsorption rates of 84% and 66% for PEF and HVED respectively). The purification yields for PEF (72 %) and HVED (60 %) samples were also lower than those of the control sample. It was explained by the different polyphenols proportions (HPLC analysis) in untreated and treated samples resulting from the extraction selectivity by PEF and HVED. The presence of polymeric polyphenols (extracted by HVED and/or PEF) could also be responsible for these lower efficiencies. The improvement of these results was suggested by choosing another adsorbent type such as Amberlite^{TM} which is widely used at the industrial scale.

7. INFLUENCES ON THE FINAL POLYPHENOLS POWDER CHARACTERISTICS

The drying process of purified extracts is usually performed at the industrial scale by freeze-drying or spray-drying. The reduction of water content would thus help keeping active the polyphenols extracts for longer time. Note that for many products, the drying step can't be performed without previous purification of extracts: the presence of sugars and proteins can form complexes or gels with polyphenols, preventing the removal of water.

The characteristics of two polyphenols powders, the first one from HVED treated samples and the second from a commercially available polyphenols powder, were compared [16]. Both powders were obtained from grape seeds. Note that in this study, the purification of extracts was performed on silica adsorbent. The color of these powders was different. The powder from HVED treated samples had a bright red-pink color. The presence of phenolic pigment predominated here. On the contrary, the commercial powder had a dark red color. This color probably resulted from the drying method used (ie spray-drying). Unlike the commercial powder, experimental freeze-dried powders had a very airy and porous structure. The experimental powders had particle size much smaller (average diameter of about 10 microns) compared to commercial powders (average diameter of about 100 microns). The polyphenols content of both powders was 99% for the commercial one and 80% for the experimental one. The previous purification step on adsorbent still had to be optimized in order to eliminate all or most of the impurities. However, in terms of polyphenols composition, the HPLC profiles of these two types of powders were similar with the dominance of two compounds: catechin and epicatechin. The production of powder mainly allows increasing the shelf life of polyphenols, in particular at room temperature. However, the powders are often redissolved for their technological applications as is the case for the wine industry. The determination of their solubility in aqueous-alcoholic solvents is therefore needed. Whatever the origin of the powder (commercial or experimental), its solubility increased with the ethanol content in the solvent. Thus, a maximum solubility was obtained for ethanol content of 25% and for pure ethanol, respectively, for the experimental and the commercial powder. The experimental powder had thus solubility greater than that of commercial powders. This result was explained by the differences in powders porosity. Indeed, the experimental powder obtained by freeze-drying had a more porous structure and a smaller particle size which facilitate the penetration of the solvent into the solid and thus its solubilization. On the other hand, the solubility of the polyphenols also depends on their average degree of polymerization (DPm). Tannins with low DPm (<6) are soluble in ethanol and methanol, those with higher DPm (> 6) are

soluble in water-acetone mixture (25:75, v / v) [49]. The commercial and experimental powders were both soluble in ethanol and have low tannins DPm: 2.3-4.6 (commercial powder) and 1.5-2.4 (experimental powder). These results indicate that the powder from HVED treated grape seeds has lots of advantages and similarities with the commercial powder. The HVED technique is thus promising for its application at the industrial scale.

8. CONCLUSION

PEF and HVED have been shown to be efficient and effective techniques for polyphenols extraction. A large range of raw materials have been tested thus confirming the feasibility of application of these technologies. But the use of PEF or HVED could be more suitable for specified products. For example, HVED is more convenient for oilseed residues and other dried products treatment. However, the purification process seems to be easier with PEF as it does not damage the product structure while HVED application often results on the product fragmentation. The final polyphenols powder had acceptable characteristics thus pointing out the potential use of these technologies at the industrial scale.

ACKNOWLEDGMENT

The authors appreciate the financial support from Conseil Régional de Picardie (France) and Fonds européen de développement régional (FEDER).

REFERENCES

[1] Kondakova V, Tsvetkov I, Batchvarova R, Badjakov I, Dzhambazova T, Slavov S. Phenol Compounds - Qualitative Index in Small Fruits. Biotechno. & Biotechnological Equipment 2009; 23: 1444-1448.

[2] Shi J, Yu J, Pohorly JE, Kakuda Y. Polyphenolics in Grape Seeds. Biochemistry and Functionality. J Med Food 2003; 6: 291-299.

[3] Escarpa A, Gonzalez MC. An overview of analytical Chemistry of Phenolic Compounds in Foods. Critical Rev Anal Chem 2001; 31: 57-139.

[4] Spigno G, De Faveri DM. 2009. Microwave-assisted extraction of tea phenols: A phenomenological study J Food Eng 93: 210-217.

[5] Heinz V, Knorr D. Effect of pH, ethanol addition and high hydrostatic pressure on the inactivation of Bacillus subtilis by pulsed electric fields. Innovative Food Sci Emerging Techno 2000; 1: 151-159.

[6] Vatai T, Skerget M, Knez Z. Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide J Food Eng 2009; 90: 246-254.

[7] Chemat S, Lagha A, AitAmar H. Comparison of conventional and ultrasound-assisted extraction of carvone and limonène from caraway seed. Flavour and Fragrance Journal 2004; 19: 188-195.

[8] El-Belghiti K, Moubarik A, Vorobiev E. Aqueous extraction of solutes from fennel (foeniculum vulgare) assisted by pulsed electric field. J Food Process Eng 2008; 31: 548-563.

[9] Gros C, Lanoisellé J.L, Vorobiev E. Towards an Alternative Extraction Process for Linseed Oil. Chemical Eng Res Des 2003; 81: 1059-1065.

[10] Sale AJH, Hamilton W. Effects of high electrical fields on microorganisms - II mechanism of action of the lethal effect. Biochimica et biophysica acta 1967; 148: 789-800.

[11] Heinz V, Alvarez I, Angersbach A, Knorr D. Preservation of liquid foods by high intensity pulsed electric fields - Basic concepts for process design. Trends in food science and technology 2002; 12: 3-4.

[12] Sigler J, Schultheiss C, Kern M. Maischeporation-ein neuer Weg der Weinbereitung. Schweiz. Z. Obst-Weinbau, 2005; 14-16.

[13] Lebovka NI, Bazhal MI, Vorobiev E. Estimation of characteristic damage time of food materials in pulsed-electric fields. J Food Eng 2002; 54: 337-346.

[14] Barsotti L. Effets des champs électriques pulsés sur les caractéristiques biochimiques et physico-chimiques de constituants et structures protéiques alimentaires. Proc of Génie des Procédés 2000; Université de Montpellier II.

[15] Zimmermann U, Pilwat G, Riemann F. Dielectric Breakdown on Cell Membranes Biophys J 1974, 14: 881-899.

[16] Boussetta N. Thesis: Intensification de l'extraction des polyphénols par électrotechnologies à partir de sous produits de la vinification 2010, Compiègne, France.

[17] Touya G, Reess T, Pecastaing L, Gibert A, Domens P. Development of subsonic electrical discharges in water and measurements of the associated pressure waves. J Phy D: Applied Physics 2006; 39: 5236-5244.

[18] Aka-ngnui T, Beroual A. Bubble dynamics and transition into streamers in liquid dielectrics under a high divergent electric field. Journal of applied Physics D Applied Physics 34: 2001;, 1408-1412.

[19] Hofmann J, Weise THGG. Pulsed power technologies for commercial material reduction and crushing applications. 11th IEEE Pulsed Power Conference 1997; 203-207.

[20] Jones H.M, Kunhardt E. E. Development of pulsed dielectric breakdown in liquids. J Physics D Applied Physics 1995; 28: 178-188.

[21] Gavrilov IM, Kukhta VR, Lopatin VV, Petrov PG. Dynamics of prebreakdown phenomena in a uniform field in water. IEEE Transactions on Dielectrics and Electrical Insulation 1994; 3: 496-502.

[22] Kukhta VR, Lopatin VV. Discharge propagation in water in non-uniform field. 12th International Conference on Conduction and Breakdown in Dielectric Liquids 1996; 259-262.

[23] Kukhta VR, Lopatin VV. Inception and development prebreakdown cavities in water. Proceedings of 13th International Conference on Dielectric Liquids 1999: 273-275.

[24] Sun B, Sato M, Clements J. Optical study of active species produced by a pulsed streamer corona discharge in water. J Electrostatics 1997; 39: 189-202.

[25] Vorobiev E, Lebovka N. Pulsed electric fields induced effects in plant tissues: fundamental aspects and perspectives of application. In: E. Vorobiev, N. Lebovka (Eds.), Electrotechnologies for Extraction from Food Plants and Biomaterials. Springer New York 2008; 39-82.

[26] Ribeiro-Sarkis J. Thesis: Extraction of bioactive compounds from seed and nut cakes and application ofelectrotechnologies on sesame, Universidade Federal do Rio Grande do Sul 2014; Brazil.

[27] Rajha HN, Boussetta N, Louka N, Maroun RG, Vorobiev EA comparative study of physical pretreatments for the

extraction of polyphenols and proteins from vine shoots. Food Research International, in press - corrected proof, 2014; (doi: 10.1016/j.foodres.2014.04.024).

[28] Boussetta N, Turk M, De Taeye C, Larondelle Y, Lanoisellé J.L, Vorobiev E. Effect of high voltage electrical discharges, heating and ethanol concentration on the extraction of total polyphenols and lignans from flaxseed cake. Industrial Crops and Products, 2013; 49: 690-696,

[29] Boussetta N, Lanoisellé JL, Bedel-Cloutour C, Vorobiev E. Extraction of polyphenols from grape pomace by high voltage electrical discharges: effect of sulphur dioxide, freezing process and temperature. J Food Eng 2009; 95: 192-198.

[30] Boussetta N, Vorobiev E, Deloison V, Pochez F, Falcimaigne-Cordin A, Lanoisellé JL. Valorization of grape pomace by the extraction of phenolic antioxidants: application of high voltage electrical discharges. Food Chem 2012; 128(2): 364-370.

[31] Boussetta N, Lebovka N, Vorobiev E, Adenier H, Bedel-Cloutour C, Lanoisellé JL. Electrically Assisted Extraction of Soluble Matter from Chardonnay Grape Skins for Polyphenol Recovery. J Agric Food Chem 200; 57(4): 1491-1497.

[32] Grimi N, Vorobiev E, Lebovka N, Vaxelaire J. Solid-liquid expression from denaturated plant tissue: Filtration-consolidation behavior. Journal of food engineering 2010; 96 (1): 29-36.

[33] Loginova KV, Lebovka NI, Vorobiev E. Pulsed electric field assisted aqueous extraction of colorants from red beet. Journal of Food Engineering 2011; 106: (2), 127-133.

[34] Lebovka NI, Mhemdi H, Grimi N, Bals O, Vorobiev E. Treatment of potato tissue by pulsed electric fields with time-variable strength: Theoretical and experimental analysis. Journal of Food Engineering 2014; 137: 23-31.

[35] Grimi N, Lebovka NI, Vorobiev E, Vaxelaire J. Compressing behavior and texture evaluation for potatoes pretreated by pulsed electric field. Journal of texture studies 2009; 40 (2): 208-224.

[36] Boussetta N, Grimi N, Lebovka NI, Vorobiev E. “Cold” electroporation in potato tissue induced by pulsed electric field. J Food Eng 2013; 115(2): 232-236.

[37] Grimi N, Mamouni F, Lebovka N, Vorobiev E, Vaxelaire J. Acoustic impulse response in apple tissues treated by pulsed electric field. Biosystems engineering 2010; 105 (2): 266-272.

[38] Grimi N, Lebovka NI, Vorobiev E, Vaxelaire J. Effect of a pulsed electric field treatment on expression behavior and juice quality of chardonnay grape. Food Biophysics 2009; 4 (3): 191-198.

[39] Zhu Z, Bals O, Grimi N, Ding L, Vorobiev E. Better damage of chicory tissue by combined electroporation and ohmic heating for solute extraction. Food and Bioproducts Processing, 2014; In press.

[40] Loginova KV, Shynkaryk MV, Lebovka NI, Vorobiev E. Acceleration of soluble matter extraction from chicory with pulsed electric fields. Journal of Food Engineering 2010; 96, 3: 374-379.

[41] Boussetta N, Lesaint O, Vorobiev EA study of mechanisms involved during the extraction of polyphenols from grape seeds by pulsed electrical discharges, Inn Food Sci Emerg Techn 2013; 19: 124-132.

[42] Boussetta N, Vorobiev E, Reess T, De Ferron A, Pecastaing L, Ruscassié R *et al*. Scale-up of high voltage electrical discharges for polyphenols extraction from grape pomace: effect of the dynamic shock waves, J. Innovative Food sci. Emerging Techno 2012; 16: 129-136.

[43] Boussetta N, Soichi E, Lanoisellé J.-L, Vorobiev E. Valorisation of oilseed residues: extraction of polyphenols from flaxseed hulls by pulsed electric fields, Industrial Crops and Products 2014; 52: 347-353.

[44] Díaz-Reinoso B, Moure A, Domínguez H, Parajó JC." Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace." Journal of Food Engineering, 2009; 91(4): 587-593.

[45] Crespo JG, Brazinha C. Membrane processing: Natural antioxidants from winemaking by-products. Filtration & Separation 2010; 47(2): 32-35.

[46] Loginov M, Boussetta N, Lebovka N, Vorobiev E. Separation of polyphenols and proteins extracted from flaxseed hull by coagulation and ultrafiltration, Journal of Membrane Science, 2013; 442: 177-186.

[47] Liu D, Vorobiev E, Savoire R, Lanoisellé JL. Intensification of polyphenols extraction from grape seeds by high voltage electrical discharges and extract concentration by dead-end ultrafiltration. Separation and Purification Technology 2011; 81(2): 134-140.

[48] Laorko A Li Z, Tongchitpakdee S, Chantachum S, Youravong W. Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice. Journal of Food Engineering, 2010; 100(3): 514-521.

[49] Karchesy J, Bae Y, Chalker-Scott L, Helm RF, Yeap Foo L. Chromatography of proanthocyanidins. In: R.W. Hemingway and P. E. Laks (Eds.) Plant polyphenols: synthesis, properties, significance, 1992; New-York.

Received on 9-07-2014 Accepted on 26-07-2014 Published on 15-1-2015

http://dx.doi.org/10.15379/2408-9826.2015.02.01.1