Recovery of Primary and Secondary Plant Metabolites by Pulsed Electric Field Treatment

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Abstract

Extraction of bioactive compounds or secondary plant metabolites safely and efficiently is one of the problems for the food industry. Moreover traditional methods, such as Soxhlet extraction, which have been used for many decades, are very time consuming and require relatively large quantities of solvents. And hence there is a need for an extraction technology which has short extraction time and reduced organic solvent consumption. In recent years several extraction techniques have been proposed. And in the quest of more efficient method for industrial production pulsed electric field (PEF) extraction technique has emerged as a potential technology. This technique is based on the conventional pulsed electric field (PEF), and provides higher electric field intensity and special continuous extraction system, which has led to reduced extraction time, higher extraction yield, and mild processing temperatures and has minimal effect on heat-sensitive biomolecule and nutritional properties. Moreover the application of moderate pulsed electric fields requires low energy inputs and may even induce the generation of certain bioactive compounds when treating metabolically active tissues. PEF extraction technique is a green technology, waste-free technology, cost-effective as well as an energy efficient process. This chapter is devoted to introduce the recent achievement of PEF extraction techniques for plant-based materials. The key focus will be on extracting large molecules, i.e., proteins, peptides, polysaccharides.

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Introduction

Extraction processes have been used since the discovery of fire. Egyptians and Phoenicians, Jews and Arabs, Indians and Chinese, Greeks and Romans, all utilized innovative extraction and distillation for processing of perfumes or food. These days, one cannot find a production line in the food industry that does not use extraction processes (e.g., maceration, steam distillation, cold pressing, squeezing, etc.). Increasing energy costs and the effort to reduce carbon dioxide emissions, food industries around the world are under a challenge to find new process technologies in order to reduce energy consumption, to meet legal requirements on emissions, product/process safety and control, and for cost reduction and increased quality as well as functionality. "Extraction process is based on the discovery, mining and design of extraction processes which will significantly reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality product."

Bioactive compounds or their precursors (antibiotics, chemopreventive agents, alkaloids, etc.) are extracted by the pharmaceutical industry, either with conventional methods or modern technologies. Recent trends in extraction techniques have largely focused on finding solutions that minimize the use of solvents. This, of course, must be achieved while also enabling process intensification and a cost-effective production of high quality extracts. How to extract bioactive compounds safely and efficiently is one of the problems for food and pharmaceutical industry. In recent years, several novel extraction techniques have been proposed. To pursuit a more efficient method for industrial production, high intensity pulsed electric field (HIPEF) extraction technique has been developed by various scientists (Yan et al. 2016). HIPEF extraction technique, which is based on the conventional pulsed electric field (PEF), provides higher electric field intensity and special continuous

extraction system, has been confirmed less extraction time, higher extraction yield, and mild processing temperature. This innovative technique is promising for application of industrial production (Yan et al. 2016).

Pulsed electric field (PEF) is one of the innovative techniques of extraction, which has obtained increasing interest in recent years because of its economic efficiency in food and pharmaceutical industry. PEF technique is originally utilized as a nonthermal technique, using short pulses of electric field to inactivate most microorganisms and some enzymes at room temperatures, in order to preserve and improve the quality of food and medicinal material (Zhao et al. 2009; Wu et al. 2014; Boulaaba et al. 2014). More recently, PEF is also applied to enhance extraction of sugars and other cellular content from plant cells, such as sugar beets.

High intensity pulsed electric field (HIPEF) processing involves the application of pulses of high voltage (typically between 20–80 kV/cm) placed between two electrodes (Yin et al. 2007). Different from PEF at low intensity level, the process of HIPEF induces the synthetic effect including strong physical and chemical reactions, which is still not fully understood in depth as of now, to increase the permeability of the membrane causing cell inactivation and enhance release of intracellular compounds through cell membrane and achieve especially higher efficiency and lower extraction time (Jin et al. 2011).

Some potential applications of HIPEF for the extraction of bioactive compounds, largely polysaccharides and proteins, have been reported in this chapter. This chapter is devoted to introduce some key results of PEF extraction technique, the critical process factors influencing its performance, and comparison of PEF extraction with other extraction techniques including traditional techniques.

Recovery of Plant Metabolites

Based on this phenomenon, electroporation (permeabilization of the cell membrane caused by external electric field) was studied in practical applications on various biological systems in the field of medicine and biology. Although recently the use of moderate field strength is considered in food science and technology, most of the studies were done in the range of irreversible membrane permeabilization, with the main objective to induce microbial inactivation or to facilitate extraction of specific constituents and/or to increase drying rates (▶ Chap. 137, “Stress Response of Plants, Metabolite Production due to Pulsed Electric Fields”).

It is important to note that the observed effects are difficult to compare due to different electric field systems used, different electric process parameters, and insufficiently described equipment and/or treatment conditions. These parameters should be standardized to enhance the evaluation of the processes described. There are several important parameters that should be concerned: electric field strength, E (kV/cm); total treatment time; number of pulses; pulse geometry; treatment chamber design; flow rate of the product (for continuous systems); fluctuation of temperature; and total energy input. More research on extraction of economically

valuable bioactive compounds has been carried out recently by various research groups. Some studies have been discussed below.

Solvent Selection and Driving Force for Pulsed Electric Field Extraction

The effectiveness of PEF in extraction of larger molecules is dependent on extraction factors such as driving force, solvents, liquid to solid ratio, etc. Several factors are important for solvent selection, i.e., the conductivity of liquid, polarity of solvent, and solubility of specific extract insolvent (Yan et al. 2016). It is well known that as the conductivity of solvent increases, the electroporation on cell membrane is enhanced, which means a probable increase in extraction rate. Therefore appropriate solvent with higher conductivity can enhance the extraction. Higher solubility of extract in solvent represents high mass transfer rate.

Solvent ratio to plant material is also important in ensuring efficient extraction in plant materials. For example, in a study on extraction of protein from beer waste brewing yeast (Liu et al. 2012), Liu et al. found an increase of protein yield at the beginning and a drop as the solvent-to-material ratio exceeded a critical value. Zhao et al. (2011) investigated the effect of solvent-to-material ratio on the extraction yield. Polysaccharide was extracted by different ratios from 20:1 to 60:1 (ml/g). The authors found that when the electric field intensity and pulse duration were fixed, the extraction yield of the polysaccharide increased from 1.8% to 6.0%. Under the process of PEF, the differential electric pressure between the cell interior and the exterior of cell membranes is significantly large that it may lead to rapid permeation. Consequently, the concentration between the cell interior and the exterior of cell membranes can reach equilibrium in a very short time. The higher the electric pressure, the more solvent can migrate into the cell and the more compounds can permeate the cell membrane (Yongguang et al. 2006). It is also believed that higher electric field strength and pulse frequency may lead to high-resonance vibration energy of materials and large self-frequency oscillation, which may cause most ionic groups in the solution to move faster and result in damage to the cellular membrane. These phenomena may be the main driving force for extraction of large molecules using PEF.

Extraction of Small Molecules, Polysaccharides, Proteins/Peptides/Amino Acids, and Lipids

Small Molecules (Polyphenols/Anthocyanins/Betanin)

Plant foods are rich sources of secondary plant metabolites such as polyphenol, anthocyanins, betanin, betalains, etc. These molecules can act as antioxidants to prevent various health problems known to human such as heart disease, inflammation, cancers, and diabetes, as well as in human cells (Ramos 2008; Hoye et al. 2008;

Wijngaard et al. 2009; Jin and Mumper 2010; Zhang et al. 2011; Sawadogo et al. 2012). The protection afforded by the consumption of plant products such as fruits, vegetables, and legumes is mostly associated with the presence of these secondary metabolites.

In recent years there has been development of newer techniques for extraction of plant secondary metabolites which include, pulsed electric field-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction. These technologies have been developed owing to the fact that they shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts (Wang and Weller 2006).

Fincan et al. (2004) reported that there was an increase in the extraction yield of betanine from beetroot following PEF treatment at 1 KV/cm with low energy consumption of 7 kJ/kg in a solid–liquid extraction compared to freezing and mechanical pressing. Ninety percent of total red coloring/betanine was released into the isotonic solution (following 1 h aqueous extraction) after applying 270 rectangular pulses of 10 μs at field strength of 1 kV/cm. The amount of pigment extracted was found to be directly proportional to the release of ionic species. PEF treatment made both the pigment and ions of the tissue equally extractable. Thus the study concluded that no differential permeabilization of any of the intracellular compartments occurred. Lopez et al. (2009) studied the effect of PEF on betanine extraction from red beetroots; they reported that the extraction yield was significantly affected by pH of the extracting media and temperature. Highest yield was reported to be at pH of 3.5 and temperature of 30 °C for betanine extraction when subjected to five pulses of 2 μs at 7 kV/cm. Moreover they also reported that mechanical pressing after PEF treatment significantly reduced the extraction time of betanine (Lopez et al. 2009). Similarly Kannan (2011) reported that following the application of PEF on red cabbage at 1 kV/cm electric field strength, 0.66 μF and 20 pulses and on red beets at 1.5 kV/cm electric field strength, 0.66 μF and 20 pulses there was a significant increase in juice extraction yield, total phenolics, anthocyanins (red cabbage), betalains (beetroots), and antioxidant activity of both red cabbage and beetroot juice compared to non-PEF-extracted samples. Interestingly they reported that there was no difference between the PEF extracted and non-PEF-extracted juice with respect to its bioactivity on cancer cells. Corrales et al. (2008) reported that PEF-assisted extraction led to improved extraction yield of anthocyanins from grape by-product. And they further reported that extraction of anthocyanin monoglucosides by PEF was better than compared to ultrasound-assisted extraction and high hydrostatic pressure extraction. The authors concluded that the use of electrical fields of 3 kV cm^{-1} may cause irreversible pores in plant membranes increasing the extractability of polyphenols by the release of solutes into the solvent and possibility of inactivating degrading enzymes which may explain the higher yields in antioxidant activity compared to other extraction techniques such as ultrasound and high pressure treatments used in the study. Lopez et al. (▶ Chap. 128, "Impact of Pulsed Electric Field Treatment on Must and Wine Quality") proposed that the application of a PEF treatment on grape skin before maceration step can reduce the duration of maceration and improve the stability of anthocyanin and

polyphenols during vinification. Similar results were reported by Delsart et al. (2012) on the PEF treatment of Merlot skin which resulted in increased extraction of polyphenols and anthocyanins. Medina-Meza and Barbosa-Cánovas (2015) reported that PEF in general give higher yield of anthocyanins, flavonols, and total phenols compared to conventional method of extraction; moreover, they also observed that larger the diameter of the chamber, more effective was the PEF treatment which may be due to higher residence time and more number of pulses. In the same line Guderjan et al. (2005) reported that the recovery of phytosterols from maize increased by 32.4% and isoflavonoidsie. Genistein and daidzein, from soybeans, increased by 20–21% when PEF was used as pretreatment process. Rajhaand et al. (▶ Chap. 143, "Pulsed Electric Fields and High-Voltage Electrical Discharge-Assisted Extraction of Biocompounds from Vine Shoots") reported a significant enhancement in the extraction yield of polyphenols following PEF treatment. They further reported that pretreatments (PEF, ultrasound and high voltage electrical discharge) induce cell damage of vine shoots, and the damage degree significantly increased with energy input. Authors concluded that cellular damage induced by studied pretreatments enables extraction of low molecular weight components such as ions modifying electrical conductivity of solution. They further deduced that a threshold of cellular damage should be determined for each pretreatment, above which the enhancement of polyphenol extractions becomes significant. Aguiló-Aguayo et al. (2015) reported an enhanced yield of bioactive polyacetylenes from carrot juice with PEF pretreatment of carrot slices followed by pressurized-liquid extraction (Table 1).

Polysaccharides

Polysaccharides are generally considered macromolecules with a minimum number of 100 monosaccharides (single sugars). Polysaccharides are important organic compounds widely distributed in nature, playing an important role in the growth and development of living organisms. The most common saccharides include sugars, starch, and cellulose. Saccharides are divided into four chemical groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Saccharides have a variety of functions in living organism (Yan et al. 2016).

The polysaccharides are regarded as storage of energy (like starch and glycogen) and as structural components (like cellulose in plants and chitin in arthropods). The 5-carbon monosaccharide ribose is an important component of coenzymes (like ATP, FAD, and NAD) and some kinds of genetic molecule (like RNA).

There have been many reports about extraction of saccharides, such as boiling extraction, leaching-out extraction, ultrasonic extraction (direct ultrasonic extraction and ultrasonic-assisted extraction), alkali extraction, enzyme extraction, microwave extraction, and supercritical fluid extraction (Yin et al. 2007; Zhao et al. 2011; Liu et al. 2012; Yan et al. 2016). The above extraction techniques have major drawbacks as they have to sustain long extraction treatment times, low extraction yield, high consumption of solvent, and high extraction temperature which may damage

Table 1 Pulsed Electric Field extraction of biomolecules from plants. ↑ increase, ↔ no significant change

Compound	Product	Treatment conditions	Effects	Reference
Bioactive compounds: Polyphenols				
Polyphenols	Rapeseed	5 kV/cm, 60 pulses 7 kV/cm, 120 pulses	Polyphenol content ↑	Guderjan et al. (2007)
Anthocyanins	Papaya seeds	40 kV/cm, 1–2000 pulse	Anthocyanin recovery	► Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues"
Isoflavonoids	Soybeans	1.3 kV/cm, 50 pulses, 1.857 kJ/kg 1.3 kV/cm, 20 pulses, 0.743 kJ/kg	Isoflavonoiddaidzein↑ (20%) Isoflavonoidgenistein (21%)	Guderjan et al. (2005)
Betalain	Beetroot tubers (*B. vulgaris*)	1 kV/cm, 270 pulses, 7 kJ/kg	Betalains recovery ↑	Fincan et al. (2004)
Total phenol	Papaya seeds	40 kV/cm, 1–2000 pulse	Total phenol content ↑	► Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues"
Total phenol	White button mushroom (*Agaricusbisporus*)	12.4 kV/cm, 24.8 kV/cm and 38.4 kV/cm, 272 μs 400 Hz and 800 Hz	Total phenol recovery (up to 51%)	Xue and Farid (2015)
Antioxidants	Rapeseed	5 kV/cm, 60 pulses 7 kV/cm, 120 pulses	Antioxidant capacity ↑	Guderjan et al. (2007)
Antioxidant	Microalgae *Nannochloropsis*	20 kV/cm, 0.01–6 ms, 1–600 pulse	Antioxidant capacity ↑	Pulsed Electric Fields and High Voltage Electrical Discharge-Assisted Selective Extraction of Biocompounds from *Nannochloropsis sp.*
Antioxidant	Papaya seeds	40 kV/cm, 1–2000 pulse	Antioxidant capacity ↑(up to 20%)	► Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues"

(*continued*)

Table 1 (continued)

Compound	Product	Treatment conditions	Effects	Reference
Lipid recovery				
Oil	Maize germ	0.6 kV/cm, 0.62 kJ/kg 7.3 kV/cm, 91.4 kJ/kg	Higher oil yield↑ (up to 88.4%)	Guderjan et al. (2005)
Oil	Olives	0.7 kV/cm, 30 pulses and 1.3 kV/cm, 100 pulses	Oil yield↑ (6.5%–7.4%)	Guderjan et al. (2005)
Oil	Soybeans	1.3 kV/cm, 50 pulses, 1.857 kJ/kg 1.3 kV/cm, 20 pulses, 0.743 kJ/kg	Oil yield ↑ (20–21%)	Guderjan et al. (2005)
Tocopherols	Rapeseed	5 kV/cm, 60 pulses 7 kV/cm, 120 pulses, 30 μs	Tocopherol content ↑	Guderjan et al. (2007)
Fatty acids	Grape juice	35 kV/cm 4 μs, 1000 pulse	Fatty acids ↔	Garde-Cerdan et al. (2007)
Lipid	Microalgae	2.7 kV/cm, 21 × 100 μs pulses, 10 Hz	Lipid yield ↑	Flisar et al. (2014)
Lipid	Microalgae *Heterochlorellaluteoviridis*	0–180 V, 60 Hz	Lipid yield ↔	Jaeschke et al. (2016)
Oil	Sesame seeds	20 kV/cm, 10 μs, 0.5 Hz	Oil yield ↑ (4.9%)	▶ Chap. 144, "Application of Pulsed Electric Energies for Oil and Polyphenol Extraction from Sesame Cake and Sesame Seeds".
Monounsaturated fatty acid	Brown rice	2 kV/cm and 1000 pulses, 100 μs, 5 Hz	Palmitic and oleic acids↑	Quagliariello et al. (2016)
Polyunsaturated fatty acid	Brown rice	2 kV/cm and 1000 pulses 100 μs, 5 Hz	Linoleic acid↑	Quagliariello et al. (2016)
Saturated fatty acid	Brown rice	2 kV/cm and 1000 pulses 100 μs, 5 Hz	Butyric, lauric, myristic, and stearic acid↑	Quagliariello et al. (2016)

Protein recovery				
Amino acids	Grape juice	35 kV/cm 4 μs, 1000 pulse	Amino acids↔	Garde-Cerdan et al. (2007)
Protein	Vine shoots	13.3 kV/cm, 0–1500 10 μs, 0.5 Hz,	Protein yield ↑	► Chap. 143, "Pulsed Electric Fields and High-Voltage Electrical Discharge-Assisted Extraction of Biocompounds from Vine Shoots"
Protein	Papaya peel	40 kV,0.1–10 μs, 400 pulse	Rate of protein extraction↑	► Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues"
Protein	Rapeseed stems and leaves	20 kV/cm,20–100 pulse, 200 ms	Protein yield↑ (up to 80%)	► Chap. 146, "Polyphenol and Protein Extraction from Rapeseed Stems and Leaves Assisted by Pulsed Electric Fields"
Protein	Microalgae *Nannochloropsis*	20 kV/cm, 0.01–6 ms,1–600 pulse	Protein yield ↑	Pulsed Electric Fields and High Voltage Electrical Discharge-Assisted Selective Extraction of Biocompounds from *Nannochloropsis sp*.
Protein	Papaya seeds	40 kV/cm, 1–2000 pulse	Protein yield ↑	► Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues"
Protein	Sesame cake	13.3 kV/cm,10 μs, 0.5 Hz	Rate of protein extraction ↑	► Chap. 144, "Application of Pulsed Electric Energies for Oil and Polyphenol Extraction from Sesame Cake and Sesame Seeds".
Protein	White button mushroom (*Agaricusbisporus*)	12.4 kV/cm, 24.8 kV/cm and 38.4 kV/cm, 272 μs 400 Hz and 800 Hz	Protein recovery↑ (up to 49%)	Xue and Farid (2015)
Others				
Phytosterol	Maize germ	0.6 kV/cm, 0.62 kJ/kg, 7.3 kV/cm, 91.4 kJ/kg	Phytosterols amount (up to 32.4%)	Guderjan et al. (2005)

some heat-sensitive ingredients in the form of inactivation and degeneration (Yan et al. 2008).

PEF extraction method works at ambient temperature, and the thermal effect in extraction process can be ignored. It processes rapidly (a few microsecond) with low volume of solvents, achieves a recovery much higher than others under the optimal parameter settings (▶ Chap. 141, "Application of Pulsed Electric Field for Treatment of Fish and Seafood"). For example, Yongguang et al. (2006) implemented the PEF continuous extraction technique for the extraction of polysaccharide from Rana temporariachensinensis David, and compared it with conventional extraction techniques, including alkali extraction method, enzyme extraction method, and compound extraction method. They observed that PEF method at electric field intensity (10–30 kV/cm), pulse duration (2–6 μs), and concentration of distilling solvent (0–1% KOH, v/v) led to 1.7 times higher extraction yield than the compound extraction method for 6 h and the product contents were more than ≈26% of that for the conventional extraction method, and the impurity of extraction material was minimal. PEF exerts its effect primarily by causing destruction of the membranes of cell. Under high electric field strength, more solvent enters into the cell and more compounds can permeate the cell membrane quite easily. The differential electric field strength between the inner and the exterior of the cell makes electropolar perforation of plant cell membrane. So increasing the PEF intensity could increase the extraction ratio of polysaccharides.

Zhao et al. (2011) studied and optimized the extraction conditions for polysaccharide from corn silk using PEF and compared it with hot-water extraction and microwave-assisted extraction. They observed that the extraction yield of PEF was 7.31% ± 0.15%, hot-water 5.46%, and microwave-assisted 6.18%. The results showed that PEF could be applied to extract value-added products from foods and agricultural matrix. Besides, the PEF method has accelerated the extraction speed and obviously promoted the yield of polysaccharide extraction from corn silk. Higher electric field strength and pulse frequency will lead to high-resonance vibration energy of materials and large self-frequency oscillation, which may cause most ionic groups in the solution to move faster and result in damage to the cellular membrane. In general, the higher the electric pressure that exists, the more violently electrons and ions will bounce, and more solvent can enter into the cell, while more compounds can permeate throughout the cell membrane. In such a case, an extraction procedure would be completed rapidly.

Jin et al. (2011) studied PEF extraction of trehalose from beer waste brewing yeast and compared with microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). The extraction rate of PEF was 103.15 μg/s, which was 15.96 times higher than MAE (6.46 μg/s) and 34.08 times higher than UAE (3.03 μg/s). Therefore, PEF represents a valuable alternative to the other unconventionally continuous extraction methods for the efficient extraction of trehalose from beer waste brewing yeast.

It has been recently demonstrated that PEF-assisted aqueous extraction of inulin from chicory tissues at E = 100–600 V/cm was evaluated (Loginova et al. 2010). Inulin, which is a valuable component, is used as a dietary fiber source or sugar

substitute in tablets. The highest effective diffusion coefficients were observed for both untreated and PEF-treated slices at high temperatures (60–80 °C) while diffusion was noticeably higher for PEF-treated than for untreated slices at lower temperatures.

Hence the PEF extraction technique has advantage of very less extraction time, higher extraction yield and extraction rate, enhancement of effectiveness of some extracts at low temperature, and high stability of bioactive components. In summary, the PEF extraction technique is an efficient alternative in comparison to conventional techniques.

Proteins, Peptides, and Amino Acids

The main driving force for protein extraction could be the accumulation of the charge in the two peaks of plant cells, which could lead to the electric potential difference of permeability and decomposition of cell structures. Therefore, more and more pores could be formed in cell membrane. As a result, the permeability of cell membrane can be improved which can aid in extraction of proteins. Though few studies have reported the changes in extraction yield of these biomolecules Raso et al. (2016) studied the application of pulsed electric fields (PEF) to the fresh biomass of *Arthrospira platensis* (a water soluble protein) in order to enhance the extraction of C-phycocyanin into aqueous media. The enhancement of the extraction yield of other pigments such as carotenoids and chlorophylls from PEF electroporated microalgae has been observed by other authors (Luengo et al. 2014). The lipophilic properties of these pigments required the use of organic solvents such as ethanol. It was observed that no extraction occurred at the initial treatments. Some authors have discovered that lower treatment times can yield higher extractions of small molecules (ions, carbohydrates, proteins, carotenoids, or chlorophylls). This behavior may be related to the extracted compounds molecular weight and to the size of the pores formed by PEF treatment. It is well known that low molecular weight compounds could cross the cytoplasmatic membrane immediately after their electroporation; the exit of molecules of higher molecular weight may require that the pores created by PEF treatment enlarge themselves in the course of time (Raso et al. 2016). The authors discovered PEF's potential for the selective recovery of C-phycocyanin from fresh biomass of *A. platensis* using water as solvent. The purity of the C-phycocyanin extract obtained from electroporated cells was higher than that obtained using other techniques based on the complete destruction of the cells.

In a different study, Garde-Cerdan et al. (2007) reported that PEF treatments applied to the grape juice did not affect the total concentration of amino acids; however, in PEF-treated grape juice, the concentrations of histidine, tryptophan, asparagine, phenylalanine, and ornithine were superiors than in the control. The possible mechanism suggested by authors was that amino acids were probably released through the pores formed in the plasmatic membrane of the grape juice indigenous yeasts, and they further suggested that the conditions of PEF treatment could cause organelle disruptions, so vacuoles were destroyed too, allowing the

proteases to have free access to the cytoplasmic enzymes; these proteases could cause cellular protein degradation into smaller peptides and amino acids.

Rajhaand et al. (▶ Chap. 143, "Pulsed Electric Fields and High-Voltage Electrical Discharge-Assisted Extraction of Biocompounds from Vine Shoots") observed higher protein yield with higher PEF energy inputs, and they concluded that for each pretreatment there exists some energy threshold, which should be overcome to extract the proteins from vine shoots. Parniakov et al. (▶ Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues") reported a significant enhancement of extraction of protein following PEF treatment compared to conventional extraction from papaya peels, and these changes were attributed to the elevation of temperature in the course of PEF treatment from 20 °C to 35 °C. Moreover they suggested that PEF may lead to "electroporation," or "electropermeabilization" of biological cells whereby the biological membrane may be electrically pierced under the effect of very short duration and large pulse amplitude (Pakhomov et al. 2010). Later Parniakov et al. (▶ Chap. 148, "Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues") conducted a two-stage procedure PEF treatment (n = 1–300) at the first stage, and supplementary aqueous extraction (+SAE) was done at T = 50 °C and pH = 7. They observed that supplementary aqueous extraction after PEF pretreatment had a significant positive effect on protein recovery from papaya seeds compared to common aqueous extraction. The authors attributed this phenomenon to cell permeabilization effect induced by PEF which facilitated the release of these components.

Parniakov et al. investigated the potential of pulsed electric field (PEF) pretreatment as a preliminary step of pH-assisted aqueous extraction of algae components from microalgae *Nannochloropsis* suspensions. They found that PEF allowed selective extraction of ionic components and water soluble proteins. Furthermore they suggested that PEF pretreatment has an excellent potential as a preliminary step of aqueous extraction of algae components and it allows selective extraction of some pure proteins. The novelty of the study was combination of PEF and pH-assisted extraction techniques, which led to selective extraction of different intracellular components. The effect of PEF at variable energy input and as a pretreatment to diffusion on the extraction of sesame cake compounds (▶ Chap. 144, "Application of Pulsed Electric Energies for Oil and Polyphenol Extraction from Sesame Cake and Sesame Seeds") were investigated and it was observed that the disintegration index and the protein contents increased with the energy inputs, until 83 kJ/kg was reached. For diffusion in different temperatures, PEF had a significant positive effect on the extraction of proteins when compared to the control sample, concluding there by that PEF can reduce the use of organic solvents and the need for high temperatures to improve diffusion. The phenomenon was attributed to the synergetic effect of PEF and thermal treatment on tissue damage of the sample, at temperatures in the range of 20–55 °C structural transitions can happen inside the tissue, facilitating electroporation. Xue and Farid (2015) studied the effect of continuous PEF treatment on the extraction of white button mushroom suspension (9% w/w). They observed that the parameters affecting the PEF extraction were mainly electric field intensity,

treatment time, and temperature. Furthermore, they found that protein concentration in the extracts increased with the increase of electric field intensity (12.4 kV/cm–38.4 kV/cm field intensity), temperature (4 °C and 20 °C), and treatment time (136–272 μs). There was a significant increase in the yield of protein from PEF extraction compared to conventional method of extraction. In conclusion PEF extraction permits high cell membrane permeabilization from white button mushroom suspension in a very short period of treatment time. Furthermore, a study was conducted on the effect of PEF-assisted extraction of proteins from rapeseed (*Brassica napus* L.) stems and leaves (▶ Chap. 146, "Polyphenol and Protein Extraction from Rapeseed Stems and Leaves Assisted by Pulsed Electric Fields"), the PEF 0.2–20 kV/cm, and was applied on fresh rapeseed stems (diameter, d = 6 mm; thickness, h = 10 mm) and leaves (surface, S = 10 × 10 mm2); however, they observed that electric field strengths (E) = 5 kV/cm did not statistically increase the proteins extraction yield, though with the application of the high electric field strength E = 20 kV/cm enhanced proteins yield (up to about 80%) was achieved. Concluding thereby that some threshold of electric field strengths should be overcome to obtain a high extraction yield of proteins from rapeseed leaf.

Lipids (Fatty Acids)

Lipids are important biomolecules as they exhibit important biological functions. Guderjan et al. (2005) investigated the effect of PEF on maize and olives for enhanced and gentle recovery of oil through induction of stress reactions. They observed that the disintegration index increased with raising moisture content, furthermore following PEF treatment, oil yield of up to 43.7% could be reached, which means a maximum increase of 88.4% of maize germ oil in comparison to the untreated samples; the phenomenon was attributed to the loss of water soluble ingredients of the germs during the rest in moisture and further the lower water content of germs after drying for 48 h (Table 1). In case of olive oil, Guderjan et al. (2005) reported that PEF showed an increase of oil yield for every pretreatment (PEF [E_{mild} = 0.7 kV/cm, 30 pulses; E_{severe} = 1.3 kV/cm, 100 pulses], a combination of freezing and thawing), compared to the reference (thermal treatment at 50 °C for 30 min) and the increase reached by PEF depended on the field strength, in the range of 6.5–7.4%. Later Guderjan et al. (2007) investigated the effect of PEF on oil yield from rape seed. They observed that oil yield of hulled and nonhulled rapeseed increased by the application of pulsed electric fields. At the electrical field strength higher than 1 kV/cm^{-1}, irreversible permeabilization was achieved and permanent pores were generated, which resulted in improved mass transfer and enhanced extraction of oil and plant ingredients. Compared to conventional extraction by Soxhlet, the yield following PEF was comparable in case of nonhulled rape seed, but was higher in case of hulled rape seed. Finally they concluded that by the application of PEF higher concentrations of total antioxidants, tocopherols, polyphenols, and phytosterols were analyzed in the oil; moreover, the oil yield increased

after pressing as well as solvent extraction. An increase in the oil yield was observed following PEF as pretreatment of sesame seeds (▶ Chap. 144, "Application of Pulsed Electric Energies for Oil and Polyphenol Extraction from Sesame Cake and Sesame Seeds"). Modifications caused in the seeds by the treatments significantly raised the oil yield when compared to a control sample. Furthermore the authors concluded that the lignan profile of the sesame oil obtained from all analyzed treatments showed the presence of sesamin and sesamolin and demonstrated that the electrical treatment did not alter this profile. The increases in oil expression reduced the amount of cake produced, though the obtained cake was rich in proteins and polyphenols.

Extraction of lipids from microalgae is a promising area without expensive algal broth pretreatment procedures, such as separation of phases and drying, and promises cheaper production of biodiesel from microalgae (Flisar et al. 2014); moreover, usage of organic solvents represents potential environment contamination with poisonous chemicals and does not enable algal regrowth after lipid extraction. Flisar et al. (2014) observed that PEF enabled a clean, cheap, quick and simple extraction of oil from microalgae cells not only for biodiesel production but also for potential highly valuable products such as pharmaceuticals and dietary products. Jaeschke et al. (2016) reported that moderate electric field (voltage intensity 0–180 V) did not give a significant high yield of lipid from *Heterochlorellaluteoviridis* microalgae, and they deduced that the intensity may not be enough to promote structural changes on the cell membrane that could enhance the extraction (Table 1). Quagliariello et al. (2016) reported that an increase in the yield of palmitic and oleic acids, linoleic acid, butyric, lauric, myristic, and stearic acid was observed following PEF treatment of brown rice. However in case of brown rice higher energy of PEF was required to get significant extraction yield which showed that the electropermeabilization of the cell membranes of ricebran is more difficult than other plants which generally have softer plant tissues.

Plant Material: Structural and Cell Size Effects

Parenchyma cells have an approximate diameter varying from 50 to 500 μm and are polyhedral or spherical in shape. Depending on the spatial arrangement and shapes of the constituent cells, parenchyma tissues can contain significant amounts of air space, which also influence texture. Intracellularly, the cells contain one or more vacuoles, different organelles, a cytoskeleton, and inclusion bodies in which important food components, such as starch, proteins, and lipids, are stored (Ranganathan et al. 2016). These components also affect the texture of the processed products. PEF treatment of solid raw materials for cell disintegration and modification of tissue structure and the effect of the treatment on microstructure and texture is the main processing goal in order to improve subsequent processing steps (▶ Chap. 76, "Techniques to Detect Electroporation in Food Tissues"). Therefore, it is the aim of this section to discuss the effect of PEF on food microstructure based on the effects of membrane permeabilization occurring after exposure of biological tissue to

an external electric field and the related release of cell content. These are the most important effects with a direct impact on food microstructure and textural properties. It is well known that PEF affects the cell membranes and thus can be expected to influence the texture of products in which the structure is largely dependent on the integrity of cells. Release of water in the extracellular space or changes in water retention properties, damage to the cell membranes, as well as a possible enhancement of enzyme reactions may occur as immediate or delayed effects. Significant structural changes occur in cell walls and plant tissues as a result of the interaction between electromagnetic fields and cellular material during pulsed electric field treatment.

The effect of pulsed electric fields (PEF) treatment on physicochemical properties of potato starch, a polysaccharide, was extensively studied by Han et al. (2009). In their investigation, potato starch–water suspensions (8.0%, w/w) were subjected to pulsed electric fields (PEF) treatment at 30 $kV \cdot cm^{-1}$, 40 $kV \cdot cm^{-1}$, and 50 $kV \cdot cm^{-1}$, respectively. The physicochemical properties of PEF-treated potato starch samples were investigated using scanning electron microscopy (SEM), laser scattering technique, X-ray diffractometry (XRD), and differential scanning calorimetry (DSC). It was concluded from SEM analysis that dissociation and damage of PEF-treated potato starch granules appeared. Some granules aggregated with each other and showed gel-like structures. It was also revealed from particle size analysis that there was an obvious increase of the granule size after PEF treatment. This has been attributed to the aggregation among granules. It was also demonstrated from other analysis that relative crystallinity, gelatinization temperatures, gelatinization enthalpy, peak viscosity, as well as breakdown viscosity of modified samples all decreased with increasing electric field strength. The SEM micrographs of native and various PEF-treated potato starch granules are shown in Fig. 1. All the results revealed that the PEF treatment can lead to an intragranular molecular rearrangement of potato starch granules, which induces changes of various physicochemical properties of the treated starch thus endowing it some new characteristics and functions.

In a different study, Blaszczak et al. (2005) suggested that the outer part of starch granule had a very dense layer which enhanced the resistance to external physical affections. Therefore, after PEF treatment, the fragments as well as the inner part of the starch granule, which had lost the protection of envelope, could absorb water more effectively and swell more easily, which result in the increase of aggregation among particles.

In a different study, Hong et al. (▶ Chap. 123, "Pulsed Electric Fields-Assisted Acetylation of Starch") studied the effect of pulsed electric fields-assisted acetylation on morphological, structural, and functional characteristics of potato starch. Pulsed electric fields (PEF)-assisted acetylation of potato starch with different degree of substitution (DS) was prepared and effects of PEF strength, reaction time, and starch concentration on DS were investigated. Results showed DS was increased from 0.054 (reaction time of 15 min) to 0.130 (reaction time of 60 min) as PEF strength increased from 3 to 5 kV/cm. The SEM study clearly revealed that the acetylated starch with higher DS had more detectable rough protrusions in external appearance. The solubility, swelling power, freeze–thaw stability, retrogradation, and light

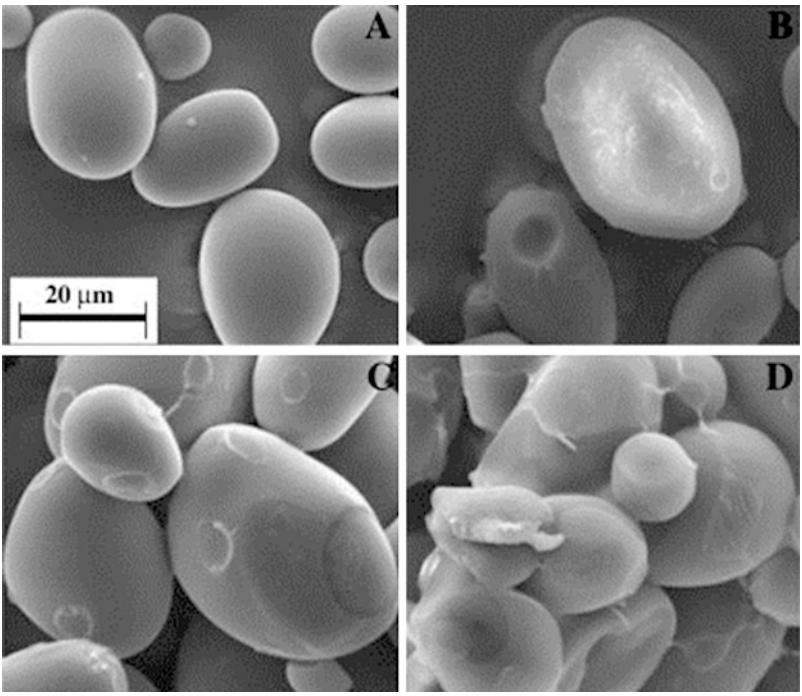

Fig. 1 SEM micrographs of potato starches treated at different electric field strengths: (**a**) native, (**b**) 30 kV·cm^{-1}, (**c**) 40 kV·cm^{-1}, (**d**) 50 kV·cm^{-1} (with permission)

transmittance of the optimal starch acetates were lower than that of the non-PEF-assisted (Fig. 2). External morphology revealed that acetylated starch with higher DS aggravated more bulges and asperities. Fourier-transformed infrared spectroscopy confirmed the introduction of acetyl group through a band at 1730 cm^{-1}. The optimum sample (DS = 0.13) had lower retrogradation (39.1%), breakdown (155 BU), and setback value (149 BU), while pasting temperature (62.2 °C) was slightly higher than non-PEF-assisted samples. These results demonstrated PEF treatment can be a potential and beneficial method for acetylation and achieve higher DS with shorter reaction time.

The exposure of biological cells to high-intensity electric field pulses can alter the structure of the cell membrane and may impact texture of foods (Goettel et al. 2013). The recovery, recycling, and sustainability of high-added value ingredients inside the food chain are an emerging topic of research. In general, the composition of plants (including seeds), bacterial and yeast cells contain lipids, glycoproteins, proteins, peptides, cellulose, and other bioactive compounds, i.e., polyphenols, etc. For example, these intracellular compounds are retained at various locations within the cell including cell wall, periplasm, plasma membrane, and cytoplasm (Balasundaram et al. 2009) in yeast. The plasma membrane, periplasmic space, and cell wall form the cell envelope, which takes about 15% of the total cell volume and serves as a

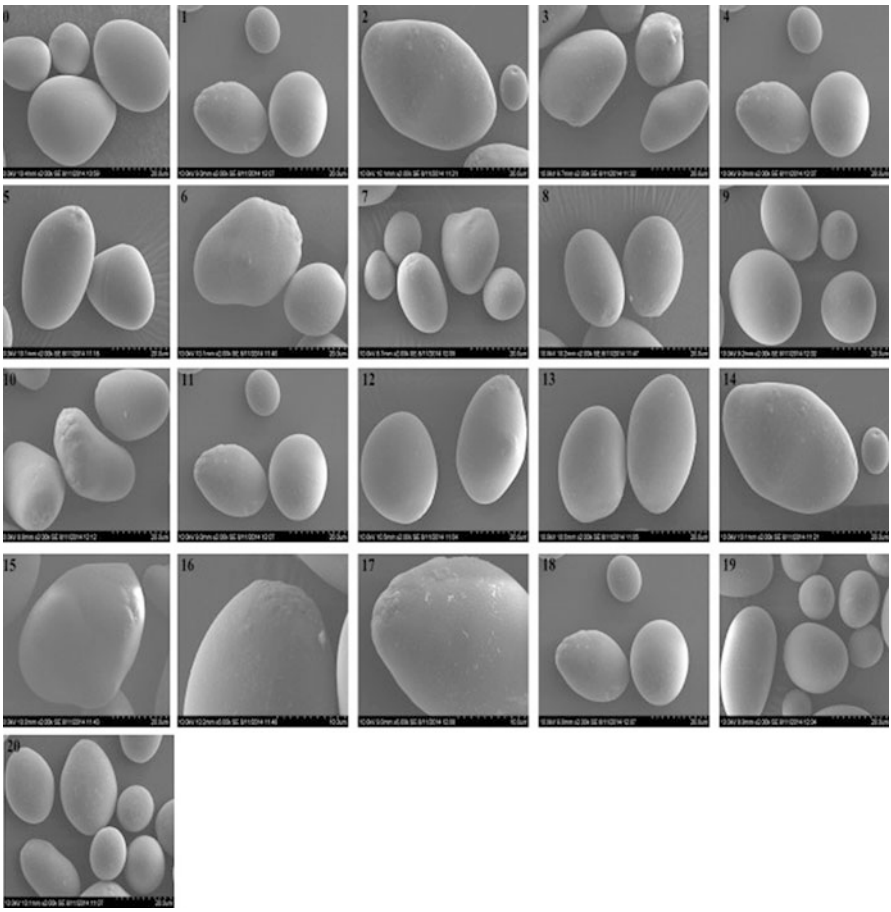

Fig. 2 SEM of native and acetylated starches treated by different electric strength. No. 0 is native starch and Nos. 1–17 are the sequence number of samples. Nos. 18, 19, 20 are control ones without PEF treatment at the starch concentration (with permission)

protecting capsule in controlling the permeability of the cell. The plasma membrane with thickness of about 7 nm separates the interior of the cell from the extracellular environment. It is a thin semipermeable lipid bilayer formed mainly by proteins and lipids, which plays a vital role in protecting the integrity of the interior of the cell by selective permeability (Feldmann 2005).Therefore, knowledge of the cell envelope structure is essential in selecting a proper disruption method and disruption condition to accommodate efficient and cost-effective release of these bioactive compounds. The disruption efficiency and selectivity of product release can be modulated by controlling electric parameters such as voltage, treatment time, applied energy, pulse waveform, and so on (► Chap. 76, "Techniques to Detect Electroporation in Food Tissues"). It has been reported that trehalose and intracellular proteins of yeast cell

can be recovered by PEF treatment (▶ Chap. 121, "Pulsed Electric Fields-Assisted Extraction of Molecules from Bacterial and Yeast Cells").

Kinetics of cell disruption and secondary metabolites release is dependent on the location. Location of an enzyme or protein and its release at different disruption condition results in the variation of the release rate of enzyme and protein. Enzymes located outside the cell membrane are generally released faster, while enzymes contained within cellular components are released at a slower rate. Primary rupture involves the breakage of the cell envelope and then the degradation of cellular debris. Several research studies (Gretchen et al. 2011; Ohshima et al. 1995) have shown that PEF can primarily cause disruption of the outer membrane relatively easily, although disruption of the inner membrane is difficult. Therefore, selective release of bioactive compounds has been achieved during PEF treatment. For example, no damage of the cell walls related to PEF application was observed by scanning electron microscopy for field strength between 0 and 18 kV/cm and number of pulses ranging within 0–500 (Ohshima et al. 1995). It is easy to release only periplasmic space material (recombinant protein) through the pores, while the co-release of contaminants and micronization of the cell debris can be minimized. The first step that takes place following PEF treatment on protein is protein unfolding; however, these structural changes can be either covalent resulting in chemically modified protein or noncovalent leading to conformational changes in protein special structures. (Manas and Vercet 2006). This unfolding of protein is the initial step for subsequent protein interaction and aggregation, which was confirmed by many researchers. Perez and Pilosof (2004) confirmed the protein aggregation and formation of covalent linked protein aggregates such as β- lactoglobulin and egg white proteins following PEF treatment. Hydrophobic interaction and disulfide bonds have been considered as the dominant binding forces involved in the formation of protein aggregate under PEF treatment, and covalent bonds other than disulfide bonds may not be involved in the protein polymerization under PEF. Hence PEF processing is effective in inactivating most endogenous enzymes in foods. However, further research from the point of biophysical chemistry of proteins is needed to obtain detailed information on the unfolding of protein molecule and the movements of amino acid residues under PEF at atomic level. Moreover, the impact of the temperature during the PEF processing on the protein structures has to be taken into account.

Conclusions

Effects of different factors for PEF extraction and optimal parameter for extraction of several types of plant materials have been discussed. Pulsed electric field presents an attractive emerging technology for the extraction of not only bioactive compounds but also other important components from the plant such as protein and lipids. One of the many advantages of PEF is that it leads to better quality of the extracted products especially in case of lipids. Moreover the extraction using PEF in no way affects the bioactivity of the components. In comparison of extraction yield and

product content with other extraction methods including conventional and unconventional methods, the results indicated that PEF extraction had advantages of higher extract yield, less treatment time, and lower energy consumption. PEF technique is promising in domain of analytical, pharmaceutical, and food industries for the near future.

Cross-References

- ▶ Application of Pulsed Electric Energies for Oil and Polyphenol Extraction from Sesame Cake and Sesame Seeds
- ▶ Application of Pulsed Electric Field for Treatment of Fish and Seafood
- ▶ Impact of Pulsed Electric Field Treatment on Must and Wine Quality
- ▶ Polyphenol and Protein Extraction from Rapeseed Stems and Leaves Assisted by Pulsed Electric Fields
- ▶ Pulsed Electric Fields and High-Voltage Electrical Discharge-Assisted Extraction of Biocompounds from Vine Shoots
- ▶ Pulsed Electric Fields-Assisted Acetylation of Starch
- ▶ Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues
- ▶ Pulsed Electric Fields-Assisted Extraction of Molecules from Bacterial and Yeast Cells
- ▶ Stress Response of Plants, Metabolite Production due to Pulsed Electric Fields
- ▶ Techniques to Detect Electroporation in Food Tissues

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