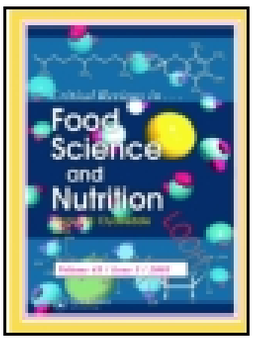

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Liang-Gong Yan, Lang He & Jun Xi (2015): High Intensity Pulsed Electric Field as an Innovative Technique for Extraction of Bioactive Compounds - A Review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1077193

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1077193

High Intensity Pulsed Electric Field as an Innovative Technique for Extraction of Bioactive Compounds - a Review

Liang-Gong Yan, Lang He & Jun Xi*

College of Chemical Engineering, Sichuan University, Chengdu 610065, China

*Corresponding author: Jun Xi, College of Chemical Engineering, Sichuan University, Chengdu 610065, China, E-mail address: xijun@scu.edu.cn

ABSTRACT

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 more efficient method for industrial production, high intensity pulsed electric field (HIPEF) extraction technique has been developed. HIPEF extraction technique, which is based on the conventional pulsed electric field (PEF), provided higher electric field intensity and special continuous extraction system, has been confirmed less extraction time, higher extraction yield, and mild processing temperature. So this innovative technique is promising for application of industrial production. This review was devoted to introduce the recent achievement of HIPEF extraction technique, including novel HIPEF continuous

extraction system, principles and mechanisms, the critical process factors influencing its performance, applications and comparison of HIPEF extraction with other extraction techniques. In the end, the defects and future trends of HIPEF extraction were also discussed.

Keywords

High intensity pulsed electric field; Bioactive compounds; Continuous extraction system; Review article.

ACCEPTED MANUSCRIPT

INTRODUCTION

Food and pharmaceutical industry presents an important area which requires the application of automation and innovative techniques to realize continuous extraction of bioactive compound and manufacture the products efficiently, reliably, cheaply, and environmentally (Wurdemann et al., 2011). To meet the requirements, many innovative extraction techniques were researched and the potential of application of them was evaluated (Puértolas et al., 2011).

Conventional extraction techniques include soaking, maceration, boiling, grinding, magnetic stirrer, water percolation, heat reflux, Soxhlet extraction, etc (Wang et al., 2006; Yang et al., 2011a; Abah et al., 2011; Azmir et al., 2013). There are more or less deficiencies and limitations in these techniques, such as long processing time, low extraction yields, high solvent consumption, poor extraction efficiency, and some of these incorporate risks of thermal degradation of thermolabile active compounds (Chan et al., 2011). To overcome the shortcomings of conventional extraction techniques mentioned above, several innovative techniques have been researched, including ultrasound-assisted extraction (UAE), pulsed electric field extraction (PEF), enzyme assisted extraction (EAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), etc (Wang et al., 2006; Azmir et al., 2013). The success of newly developed methodology was confirmed by comparing with conventional extraction methods, such as Soxhlet method (Smith, 2003).

Pulsed electric field (PEF) is one of an innovative technique 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 non-thermal technique, using short pulses of electric field to inactivate most micro-organisms and some enzymes at room temperatures, in order to preserve and improve the quality of food and medicinal material (Zhao et al., 2009; Puértolas et al., 2010; Wu et al., 2014; Boulaaba et al., 2014). The pioneer trail related to the application of PEF on extraction was carried out by Ganeva et al. (Ganeva et al., 1999). They used pulsed electric field of 2.75kV/cm to pretreat beer yeast and keep macerating for 5h to extract protein, finding that PEF technique can significantly improve the dissolution yield of protein. Further research found that biological membrane was affected by PEF which leads to enhance the mass transfer through membrane and then PEF technique attracted more scientists' interest. Various studies had examined the potential applications of this technique, for extraction of bioactive compounds, particularly, in this time most research utilize a combination of PEF technique and conventional method such as stirring to carry out extraction. (El-belghiti et al., 2005; Goettel et al., 2013; Pourzaki et al., 2013; Zbinden et al., 2013; Parniakov et al., 2014; Eduardo et al., 2015). As intensification pretreatment (PEF assisted extraction), PEF was proved to improve significantly the extraction yields of bioactive ingredients. Generally, higher electric field intensity (over 10kV/cm) was rarely adopted in PEF assisted extraction technique (Goettel et al., 2013; Parniakov et al., 2014), because there was a generation of strong hydrogen radicals

which might attack the bioactive compounds, during higher intensity PEF processing (Wu et al., 2014). Moreover, complicated operation (combined with other extraction method), low treatment capacity and discontinuous process limits its application in industrial production. Further research found that some kinds of bioactive compounds, such as bovine serum albumin, kept intact when PEF intensity exceeded 10kV/cm (Wu et al., 2014). Then PEF technique with higher electric intensity attracted more and more attention. Based on previous researchers' work, Yin et al. (Yin et al., 2006) designed an advanced continuous PEF process system, set the electric field intensity over 10kV/cm and realized efficient extraction of several kinds of bioactive compounds. High intensity pulsed electric field (HIPEF), a fast, efficient, low-energy, continuous extraction technique, was developed.

High intensity pulsed electric field (HIPEF) processing involves the application of pulses of high voltage (typically between 20 and 80 kV/cm) placed between 2 electrodes (Yin et al., 2006). 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 understood in depth 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). Compared with other non-thermal processes such as high hydrostatic pressure method, HIPEF extraction method requires much shorter processing time (a few microseconds), higher extraction yield and the technique can be easily applied in continuous

flow, which is applicable for industrial manufacture. Several materials have been extracted under HIPEF process (Yin et al., 2002; Yin et al., 2006; Yin et al., 2007; Yin et al., 2008a; Yin et al., 2008b; Zhao et al., 2011, Jin et al., 2011; Liu et al., 2012; Li et al., 2012; Lin et al., 2012; He et al., 2014). To date, some research on HIPEF technique are carried out and it is pointed that the mechanism must be better understood for industrial application.

Some potential applications of HIPEF for the extraction of bioactive compounds have been reported. This review is devoted to introduce the recent research result of HIPEF extraction technique, including novel HIPEF continuous extraction system, principles and mechanisms, the critical process factors influencing its performance, comparison of HIPEF extraction with other extraction techniques including traditional and other innovative techniques. In the end, the defects and the future trends of HIPEF extraction were also discussed.

EXTRACTION SYSTEM AND DEVICE PARAMETERS

Figure 1 shows a specially-made continuous HIPEF extraction system, which includes a high-voltage repetitive pulse generator, liquid materials treatment chambers, a thermostat, and a peristaltic pump (Yin et al., 2002; Yin et al., 2006; He et al., 2014). The circuit of pulse generator is shown in Figure 2 (Yin et al., 2008a) and form of pulsed wave is shown in Figure 3 (Jin et al., 2011). In consideration of cost and reliability, exponentially decaying bipolar triangle pulsed waveforms generator was constructed (Yin et al., 2006). The 220 volts ac was turned to kilovolt ac by transformer, and then commutated to a high-voltage dc. R1 was the limiting

current resistor avoiding arc; R2 was used to control the discharge time; R3 and capacitor C constituted charging circuit. R1, R2, C and HIPEF treatment chamber with material constituted discharge circuit. The process of charge and discharge was controlled by the switch and the switch was controlled by the high-voltage trigger generator. The frequency was adjustable ranging from 40 to 3000 Hz and pulse width was 2μs (He et al., 2014).

The detail of treatment chambers is shown in Figure 4. Each one contains two stainless steel electrodes with a gap. Annular electrodes had been used because of protection of electrodes due to avoidance of electrolysis and electrocorrosion caused by partial high intension electric field generated near the electrodes (Yin et al., 2002). One electrode was connected to pulsed waveforms generator and the other one was earthed. A small hole was cut off from the insulator layer which was made of polyisobutylene acid resin to form electric field concentration in the middle of two electrodes where HIPEF worked. In this review, the distance between electrodes was chosen 1.5mm and the radius of the hole on insulator layer was chosen 0.5mm, so the actual treatment volume was considered 1.17×10^{-3} ml (Yin et al., 2007; He et al., 2014). The pulsed waveform and input voltage could be monitored on a digital oscillograph and caught by other data acquisition equipment. Depending on the design of treatment chamber, HIPEF process can operate in either continuous or batch mode, thus fits for mass production.

The general procedures of HIPEF processing are shown in Figure 5. Before HIPEF treatment, raw material had been ground, filtered, sieved, and mixed with solvent by agitator.

And the suspended samples were transferred to treatment chamber by peristaltic pump at given flow velocity, processed by HIPEF once or more depend on the numbers of chambers. In this review HIPEF system mentioned had two treatment chambers. The temperature of solution was maintained by thermostat. After HIPEF treatment, the extract was separated from solution, cleaned, purified, dried, and determined.

Parameter of HIPEF system consists of pulse number (n) or pulse duration (t_i, μs), electric field intensity (E, kV/cm), and total treatment time (T, min).

The pulse number (n) is calculated as

$$n = \frac{l\pi r^2 f}{q} \qquad (1)$$

The electric field intensity (E, kV/cm) is calculated as

$$E = \frac{V_{p-p}}{2l} \qquad (2)$$

The total treatment time (T, min) is calculated as

$$T = \frac{Q}{q} \qquad (3)$$

The pulse duration (t_i, μs) is calculated as

$$t_i = N_c W_p n \qquad (4)$$

where 1, the distance between two electrodes of treatment chamber (mm); r, the radius of the hole on insulator layer (mm); f, the frequency (Hz); q, the flow velocity (ml/min); V_{p-p}, the voltage provided by pulse generator (kV); Q, the treatment quantity (ml); W_p, the pulse widths (μs); N_c, the number of section of treatment chambers. In this review, 1 is 1.5mm, r is 0.5mm, W_p is 2μs, N_c is 2, and other parameters were changed accordingly. This shows that there is an association between the parameters. The total treatment time decreases with the flow velocity increasing but pulse duration reduces which might weaken the extraction. Using higher frequency make pulse duration increasing which might achieve higher extraction yield. The flow velocity was fixed at a given value and pulse duration was changed by adjusting the frequency of pulse generator.

One other thing to note was that application of decaying bipolar triangle pulsed waveforms contribute to energy loss, the part below the critical field intensity do not works, so the actual PEF effective time (t_a, μs)

$$t_a < t_i \quad (5)$$

The actual PEF effective time (t_a, μs) is difficult to be achieved. In single factor test, pulse duration could be considered as an alternative to actual PEF effective time because of uniformity of wave shape.

PRINCIPLES AND MECHANISMS

The primary aim of PEF treatment is attaining microbial inactivation, mainly expressed by permeabilization of cell membrane which induced by electroporation (Donsi et al., 2010). The mechanisms of inactivation of cell under HIPEF were considered much more complex that include a multitude of physical effects and chemical reactions which were not known well (Lin et al., 2012; Liu et al., 2012). As the major effect to permeabilization, electroporation was still proposed to explain the extraction mechanisms of HIPEF treatment (Yin et al., 2007; He et al., 2014).

Electroporation is the phenomenon in which a cell exposed to high intensity electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes (Castro et al., 1993). The exact mechanisms of electroporation are not fully understood. Several theories have been proposed in order to explain the mechanism. Although, all of these theories have their own strong points and weaknesses, they reach a consensus that the membrane plays an important role in amplifying the applied electric field, when the conductivity of intact membrane is much lower than the one of extra cellular medium and cell cytoplasm. As showed in Figure 6 (Donsi et al., 2010), during suspension of living cells in electric field, an electric potential which passes through the cell membrane increases as a result of the charging process at the membrane interfaces and separates molecules according to their charge under the dipole nature of membrane molecules in the cell membrane. Here r is the radius of cell and θ is the angle

between the site on the membrane and the direction of the electric field. The highest potential occurs at the cell poles ($\theta = 0, \pi$) and decrease to 0 at $\theta = \pm\pi / 2$. Because the thickness of membrane (about 5nm) much thinner than plant cell radius (about 100μm) and the conductivity of the former much lower than the latter's, a selective concentration of the electric field on the membrane occurs, creating a strong trans-membrane electric field, which is about 10^5 times higher than applied field intensity. When the trans-membrane potential exceeds a critical value (typically 0.2-1.0V for most cell membrane), pore occurs in weak areas of the membrane and causes drastic increase of permeability. Similarly, electroporation also occurs in the liposomes but the molecules affected by electric field are not necessarily the same (Tsong, 1990). Some research suggested that initial pore forming was a response to an electrical suprathreshold potential and the expansion of the pores depend on the intensity of the electric field, pulse duration, and the ionic strength of the medium (Kinosita et al., 1977). When the size and number of pores reach the critical value related to the total membrane surface, reversible breakdown turns into irreversible breakdown, which causes perpetual mechanical destruction of the cell membrane. Measurement of cell size before and after treatment could serve as an indicator for cell vitality to confirm whether irreversible breakdown occurs (Janositz et al., 2010).

CRITICAL PROCESS FACTORS

Experiments show that effectiveness of HIPEF treatment strictly depends on these factors as follow: electric field intensity, pulsed wave shape, solvent selection, ratio of raw material to solvent, pulse duration and treatment temperature, etc.

a) Electric field intensity. Electric field intensity is one of the main factors that influent cell inactivation. As discussed above, the cell inactivation increases with applied electric field intensity increasing when the field intensity exceeds a critical value named critical field intensity. The recent research indicated that critical field intensity became larger for cells of larger size (Jeyamkondan et al., 1999). The parameter also depends on pulse width. When pulse width was greater than 50μs, the critical field intensity was 4.9kV/cm. When pulse width was less than 2μs, the value was 40kV/cm (Schoenbach et al., 1997). Generally, higher electric field intensity caused higher levels of extraction. Yin et al. (Yin et al., 2006) found that when the PEF intensity was increased from 5 to 20kV/cm, the extraction recovery of polysaccharide from *Rana temporaria chensinensis* David was increased rapidly from 17.11% to26.87%, however, with the increase in electric field intensity from 20kV/cm to 40kV/cm, the extraction recovery was decreased from 26.87% to 21.41% due to decomposition of the polysaccharide. So in this research 20kV/cm in PEF intensity was eventually chosen as optimal parameter. Similar results were reported in the extraction of chondroitin sulfate from fish bone (He et al., 2014). When the electric field intensity exceeded 15kV/cm, the growth rate of content of chondroitin sulfate

leveled off, and when the electric field intensity reached 25kV/cm, the content was maximized to 5.84g/L. In consideration of stability of system and avoidance of irreversible damage, the electric field intensity could not be infinitely increased. To sum up, a great increase of extraction yield was achieved at the forepart of increase of electric field intensity, and a slight increase even a decrease was achieved with electric field intensity exceeding a particular level. Certainly the most appropriate values should be determined under overall consideration.

b) Pulsed wave shape. Electric field pulses may be applied in the form of exponential decaying, square, oscillatory, bipolar, or instant reverse charges. Some research demonstrated that square wave pulses were more energy and lethally efficient for cell inactivation than oscillatory pulses and exponential decaying pulses (Qin et al., 1994). And bipolar pulses were more efficient than mono-polar pulses because it caused reciprocating movement of charged molecules in the cell membranes of microorganisms which causing a stress in the cell membrane and enhancing its electric breakdown (Qin et al., 1994; Ho et al., 1995). Application of bipolar pulses also took the advantages of minimum energy consumption, reduced deposition of solids on the electrode surface, and decreased the negative effect of electrolysis (Barbosa-Cánovas et al., 1999). In this review, exponentially decaying bipolar triangle pulsed waveforms was adopted and excellent results were achieved. So there is reason to believe that the HIPEF system could be improved greatly in further research.

c) Solvent selection, concentration and pH. Several factors are important for solvent selection: the conductivity of liquid, polarity of solvent, and solubility of specific extract in solvent. As mentioned above, as the conductivity of solvent increasing, the electroporation on cell membrane is enhanced, which means a probable increase in extraction rate. Thus, choosing solvent with higher conductivity may enhance the extraction with an application of equal input energy. The other hand, the solubility of specific material is important as well. High solubility of extract in solvent represents high mass transfer rate. In the research on extracting betulin from *Inonotus Obliquus* (Yin et al., 2008b), organic solvents were used because of betulin's poor solubility in water. 75% (v/v) ethanol, pure ethanol, methanol were available in this research. Due to the weak polarity of high-concentration ethanol, strong polarity of water, and enhancement of mobility of the polar molecular in solution, by PEF, the betulin extracting yield in 75% (v/v) ethanol was higher than that in pure ethanol. Although the yield in methanol was slightly higher than in ethanol, ethanol was finally selected due to non-toxic and recyclable properties. One more result was reported in the extraction of chondroitin sulfate from fish bone (He et al., 2014). With NaOH concentration increasing below 3% (v/v), the extraction yield increases fast, and when the solvent concentration is greater than a specific value of 3% (v/v), the extraction yield still increases but at a flattened speed. Therefore from the view of resource optimization, 3% (v/v) NaOH was chosen to extract chondroitin sulfate. It was noteworthy that an obvious decrease in product content occurred and the electric field gave a spark of light when

the solvent increasing to higher concentration level (Yin et al. 2006), which need investigation in further research. In addition, Jin et al. (Jin et al., 2011) studied HIPEF extraction of trehalose from beer waste brewing yeast and observed that increase of pH from 3 to 7 induced significant increase of extraction yield and reached a highest yield at pH of 7. The article did not explain this consequence.

d) Ratio of solvent to raw material. Ratio of solvent to raw material is also important in ensuring efficient extraction. Yin et al. extracted betulin from *Inonotus Obliquus* (Yin et al., 2008b) and found that extract content increased obviously from 4.1 to 6.2g/kg as the solvent-to-material ratio increased from 5:1 to 30:1 (ml/g). It was indicated that solvent to material was effective for improvement of extract yield. But if the solvent-to-material ratio increased sequentially, the extract yield stopped increasing due to the decrease of concentration of betulin in the solution. Moreover, more energy had to be consumed to remove the solvent from solution after treatment. In the research on extraction of protein from beer waste brewing yeast (Liu et al., 2012), Liu et al. found an increase of protein yield at beginning and a drop as the solvent-to-material ratio exceeded a critical value. Zhao et al. (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). When the electric field intensity and pulse duration were fixed at 30kV/cm and 6μs respectively, the extraction yield of the polysaccharide increased from 1.79%±0.27% to 6.18%±0.43% with the ratio increasing from 20:1 to 50:1. And then the

yield approached its maximum. It is suggested that the increase in the ratio of solvent to raw material could eventually dilute the concentration of product. He et al. explained that with a certain amount of materials, as the amount of solvent increased, concentration of bioactive compounds around the sample particles was reduced and the bioactive compounds were easier to dissolute as a result. But the chance of molecular collision reduced in contrary to increase of the amount of solvent. Therefore, an optimal value could be found in certain range of solvent-to-material ratio (He et al., 2014).

e) Pulse duration. Pulse duration is one of important indicators that measure PEF treatment, defined as the product of the pulse numbers and pulse width. An increase in either of pulse numbers and pulse width enhances cell inactivation (Sale et al., 1967). However it is not necessarily that longer pulse duration contributes to the optimum of extraction because of an undesirable temperature increase. So the optimum processing conditions should be established based on experimental data to obtain the highest inactivation rate with the lowest heating effect. The HIPEF method in this article fixed pulse width at 2μs in accordance with the form of pulsed wave applied and altered pulse duration by adjusting the frequency of pulse generator. The research on HIPEF extraction of polysaccharides from *Rana temporaria chensinensis* (David Yin et al., 2006) showed that a great increase in extraction yield occurred when the pulse duration increased from 2 to 6μs. However, with the increase of electric pulse duration from 6 to 8μs, the extract yield decreased in contrary. The result explained that increasing the pulse duration

enhance the permeation of cells but led to decomposition of extracts at the same time. Similar results were reported in the extraction of betulin from *Inonotus Obliquus* (Yin et al., 2008b), polysaccharides from corn silk (Zhao et al., 2011), and chondroitin sulfate from fish bone (He et al., 2014). Yin et al. (Yin et al., 2007) applied HIPEF technique to extract DNA from bovine spleen and owed the phenomenon to the varied behavior of cell membranes to PEF, which lead to membrane's protein unfolding, breakdown of covalent bonds and oxidation-reduction reactions, which become more serious as pulse duration prolonged.

f) Treatment temperature. Few articles on optimization of treatment temperature had been reported because most experiments were carried out at room temperature. The temperature to extract DNA from bovine spleen was set at 65℃ because of high efficiency. Lower temperature results in combination of protein and nucleic acid and higher temperature induces nucleic acid to degeneration (Yin et al. 2007). Generally, higher temperature decreases the viscosity of liquid solvents to strengthen penetration of matrix particles and enhances extraction. Maximum temperature is limited by degeneration temperature of active compounds of product.

APPLICATIONS AND COMPARISONS

Saccharides

Saccharides are significant 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: mono-saccharides, disaccharides, oligosaccharides, and polysaccharides. Saccharides have a variety of functions in living organism. 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). Science shows that saccharides and their derivative play important roles in immune system, fertilization, preventing pathogenesis, blood clotting, and development as well (Anthea et al., 1993). In conclusion, the improvement of extraction techniques of saccharides has a good application prospect.

Saccharides are abundant in nature resources, including plants, animals and microorganisms. 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., 2006; Zhao et al., 2011; Liu et al., 2012; Brereton et al., 2012; Zhang et al., 2014; Cardenas-Toro et al., 2014). These extraction methods mentioned above have some drawbacks more or less. Most of these methods have to sustain long extraction time, low extraction yield, high consumption of solvent, high extraction temperature which could damage some thermo-sensitive ingredients in the form of inactivation and degeneration (Yan et al., 2008,

Xi, 2013). Moreover, it is difficulty to realize continuous extraction in application of these methods. It is good news that HIPEF method overcomes the shortcomings mentioned above. HIPEF extraction method works at room temperature and the thermal effect in extraction process can be neglected. It processes rapidly (a few microsecond) with low volume of solvents, achieves a recovery much higher than others under the optimal parameter settings (He et al., 2014). It is more important that the HIPEF continuous extraction system this research employs is safe and reliable. Although there are some problems need to be solved, HIPEF continuous method promise good prospect for industrial production as a safe, efficient and environmental-friendly extraction method.

For example (Table 1 & 2), Yin et al. (Yin et al., 2006) employed the HIPEF continuous extraction method for the extraction of polysaccharide from *Rana temporaria chensinensis* David, and compared it with conventional extraction methods, including alkali extraction method, enzyme extraction method and compound extraction method. The process parameters of HIPEF method were optimized and evaluated in range of various experimental conditions, respectively, such as electric field intensity (10-30kV/cm), pulse duration (2-6μs), and concentration of distilling solvent (0-1% KOH, v/v). At the beginning, 2g of frog powder was weighted and mixed with a quantity of appropriate solvent and then the mixture was pumped through the HIPEF system with a flow velocity of 26 ml/min. In the alkali extraction method, samples were prepared by mixing 2g of frog powder with 5% (v/v) KOH at 45℃. The product

prepared to determine the polysaccharide after 2h of moderate shaking and subsequent filtering. In the enzyme extraction method, samples were prepared by mixing 2g of frog powder with pepsin at 50℃. After 5h, the products were filtered and prepared to determine the polysaccharide. In the compound extraction method, samples were prepared by mixing 2g of frog powder with 1% (v/v) KOH and compound enzymes at 50℃. After 6h, the products were filtered and prepared to determine the polysaccharide. In result, the optimal conditions of HIPEF extraction of polysaccharide were as follows: electric field intensity was 20kV/cm, pulse duration 6μs, and solvent 0.5% (v/v) KOH. The result of research showed that the largest extraction yield was 55.59% and product content was 43.15mg/L by HIPEF on the optimal condition. By comparing it with conventional extraction methods, the extraction yield and product content of HIPEF method were much higher than the other three methods the extraction yield and product content of alkali extraction method were 19.78% and 10.78mg/L, enzyme extraction method 24.44% and 14.93mg/L, compound extraction method 31.38% and 16.81mg/L. According to the data, the HIPEF extraction yield just for 6μs was 0.77 times higher than the compound extraction method for 6h and the product contents were more than 26.34% of that for the compound extraction method and the impurity of extraction material was few. So HIPEF extraction method is efficient and promising method to extract polysaccharide of *Rana temporaria chensinensis* David.

Zhao et al. (Zhao et al., 2011) studied and optimized the extraction conditions for polysaccharide from corn silk by response surface methodology based on a Box-Behnken design. Electric field intensity, ratio of raw material to liquid and pulse duration were investigated as independent variables. Sample was pumped into the HIPEF system at a flow velocity of 25ml/min. Experiments showed that optimal extraction conditions were as follows: electric field intensity 30kV/cm, ratio of liquid to raw material 50:1, and pulse duration 6μs. Comparing with hot-water extraction (Boiling) and microwave-assisted extraction (MAE), the extraction yield of HIPEF got 7.31%±0.15%, hot-water 5.46% and microwave-assisted 6.18%. The extraction time of hot-water extraction was 60min and microwave-assisted 33min (3min for microwave treatment and 30min for hot water treatment). The results showed that HIPEF could be applied to extracting value-added products from foods and agricultural matrix.

Jin et al. (Jin et al., 2011) reported that the HIPEF was applied to extracting trehalose from beer waste brewing yeast in comparison with microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). Several independent variables such as pH (3-7), electric field intensity (0-50 kV/cm), pulse numbers (0-10) and ratio of liquid to solid (20:1-60:1) were investigated. The flow velocity of material pumped into system was chosen 0.4ml/s (24ml/min). The results showed that under the optimal conditions of electric field intensity of 19.97kV/cm, pulse number of 6, and liquid-solid ratio of 30:1, the extraction yield of trehalose could reach 2.635% in 6min of total treatment time with 7.72% of UAE in 380min and 9.24% of MAE in

213min. As shown in Table 3, the extraction rate of HIPEF was 103.15μg/s, which was 15.96 times higher than MAE (6.46μg/s) and 34.08 times higher than UAE (3.03μg/s). Therefore, HIPEF represents a valuable alternative to the other unconventionally continuous extraction methods for the efficient extraction of trehalose from beer waste brewing yeast.

Li et al. (Li et al., 2012) used the HIPEF system for study of extraction of fungal polysaccharide from Jew's Ear (Auricularia auricular) and identification of the best strain to yield fungal polysaccharide with anti-coagulant activity. Three strains of Jew's Ear and three extraction techniques, HIPEF extraction, microwave-assisted extraction (MAE) and ultrasonic assisted extraction (UAE) were applied to optimizing the extraction conditions. It showed that the optimal extraction conditions of HIPEF, when treatment flow velocity was fixed at 5ml/min and treatment temperature was kept below 40℃, were as follows: electric field intensity 24kV/cm, ratio of liquid to raw material 30:1, pulse duration 12μs (pulse number at 6), and pH 8. By comparing with conventional extraction methods, it was confirmed that the HIPEF exhibited the most remarkable effect of prolongation of blood clotting time among the three methods. In result the HIPEF method greatly enhanced the anti-coagulant activities of extracts. That suggests that the HIPEF technique will be an effective method in the manufacture of bioactive natural polysaccharide.

These results mentioned above show that the HIPEF extraction technique take advantages 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 HIPEF extraction technique has a good prospect in the manufacture of saccharides

Organic calcium

The calcium is the most abundant mineral material by mass in many animals, including human and it is essential for life. The National Osteoporosis Foundation declares that calcium plays an important role in building stronger, denser bones early in life and keep bones strong and healthy later in life. In the human body, most of the calcium is stored in the bones and teeth, and the rest has other significant effectiveness such as the contribute to blood coagulation of neuromuscular excitability, cell adhesion, the transmission of nerve impulses, the maintenance of membrane function, enzyme activation and the secretion of hormones (Tang et al., 2002). Long-term calcium deficiency induces various diseases such as rickets, cancer, cardiovascular disease, diabetes, and autoimmune disorder, which might lead to osteoporosis and increases the risk of fracture especially for menopausal women. What is more serious is that a lifelong calcium deficiency can affect formation of bone and tooth, damage kidney function and decrease absorption of other minerals (Glade, 1997; Ross et al., 2011). The phenomena of calcium deficiency in human are widespread in many countries and regions now, especially undeveloped countries (Jarrett et al., 2013; Prentice, 2013). So the industry of production of calcium is promising. Calcium is abundant in the nature. Many vegetable, including spinach, chard and rhubarb, are good resource of calcium. Seaweed, nuts and beans have a high content of calcium

as well. How to extract calcium from natural materials efficiently and stably become a problem need to be solved.

Recently, Yin et al. (Yin et al., 2008) proposed to extract dissoluble calcium from bones using the HIPEF technique. The fresh bone was ground, cooked, and then deep ground, eventually dried. After that, the samples were sieved to 0.147mm. 5g±0.01mg sample above was weighted and added to citric acid, dissolved and mixed with water to 200ml. The bone solution was pumped into HIPEF system at a flow velocity of 11.775ml/min, and treated at room temperature. After being centrifuged, the clear liquid was taken to determine dissoluble calcium content in the end. Various experimental conditions of the HIPEF method, such as electric field intensity (0-70kV/cm), pulse numbers (0-12) and citric acid concentration (0-2%, v/v) were investigated, and the optimal results were compared with traditional extraction methods covering blank (solvent is water), stillness, boiling, and microwave. The results displayed that the most content of dissoluble calcium could be achieved at 4328.8mg/l in total treatment time of 8.5min by HIPEF on the condition of 1.25% (v/v) citric acid, 70kV/cm electric field intensity and 12 pulse numbers (pulse duration 24μs). The values of control methods were 22.032mg/L in 24h (blank), 210.522mg/L in 24h (stillness), 506.981mg/L in 40min (boiling), 1413.72mg/L in 15min (microwave). Moreover, the nutriments in bones solution treated by HIPEF method were remained preferably. So HIPEF is a great extraction method for extraction of calcium, non-thermal, fast, efficient and has non-negative effect.

Eggshells are good calcium resource as a nutritional, however millions tons of eggshells were wasted in China every year. Lin et al. (Lin et al. 2012) optimized HIPEF extraction method for production of eggshell calcium malate. According to the experimental design, 1g eggshell powder was weighted and mixed with 50ml malic acid solution. Then the mixture was pumped into the HIPEF extraction system at a flow velocity of 25ml/min and the pulse generator was turned on. After 2min, the product was taken out, subsequently centrifuged to remove the solid matter, and finally prepared for determination of dissoluble calcium content. Combinations of electric field intensity (5-30kV/cm), solvent concentration (1.6-8.0%, v/v), and pulse duration (4-20μs) were variables for HIPEF according to single factor test and ternary quadratic regression orthogonal combination design. Consequently, the highest dissoluble calcium malate content (7.075mg/ml) was obtained with the 6.0% (v/v) malic acid, the electric field intensity of 20kV/cm, and pulse duration of 24μs. It was proved that the HIPEF technique could be a highly effective, environmentally friendly, easily-operated, and energy-saving method for production of eggshell calcium malate, which could promote the absorption of calcium in vitro.

Proteins

Proteins, which are the essential macromolecules of life, play important roles in catalyzing the numerous biochemical reactions that are required to provide energy for organisms, and for each form of biological movement. In addition proteins perform all of the other functions of any given organism, such as photosynthesis, or for example, in animals, neural, vision, and structure

function. Most plants and microbes can biosynthesize all twenty standard amino acids but animals, of course, including humans cannot produce all kinds of amino acids by themselves. Particularly, humans can only synthesis half of the twenty standard amino acids. The other amino acids, including valine, methionine, leucine, isoleucine, phenylalanine, lysine, threonine, and tryptophan for adults, histidine and arginine for babies must be obtained through diet (Mousdale et al., 1991).

The extraction of trehalose from beer waste brewing yeasts has been discussed above. In fact, large quantity of the amino acids which are essential to human, especially lysine short in cereal protein, store in beer waste brewing yeasts (Sun et al., 2007). So, beer waste brewing yeasts are important resource of human body essential amino acids as well. Therefore the efficient exploitation and utilization of protein from beer waste brewing yeasts has great economic value. Liu et al. (Liu et al. 2012) carried the novel HIPEF technique to extract protein from beer waste brewing yeasts. The yeast paste was sieved into a particle size of 200μm, washed, and kept under 4℃until use. Except for treatment temperature and flow velocity fixed (room temperature and 0.4ml/s), the effect of other parameters such as electric field intensity (10-50kV/cm), pulse duration (2-16μs), and liquid-material ratio (20:1-60:1) were studied for the optimal extraction conditions by one-factor-at-a-time experiment and quadratic regression orthogonal design. The results showed that the extraction yield of protein could reach 2.788±0.014% when electric field intensity was 10kV/cm, pulse number was 6 (pulse duration was 12μs) and liquid-material ratio

was of 50:1. In conclusion, HIPEF was proved an advanced efficient extraction method once again. This article pointed that further work was necessary in order to identify the specific mechanism of HIPEF working on yeast cell membrane.

Other Bioactive Compounds

More research on extraction of economically valuable bioactive compounds was carried out recently. The advantages of HIPEF technique were demonstrated for several kinds of bioactive ingredients such as betulin, chondroitin sulfate, DNA, and so on (Table 1).

Betulin has protective effects against Cadmium-induced apoptosis (Oh et al., 2006) and betulinic acid has anti-malarial, anti-inflammatory, anti-HIV, and anti-tumor effectiveness (Kvasnica et al., 2005). So the extraction of betulin and butulinic acid intrigued the scientists. The good news is that Betulin can be easily converted to betulinic acid (Jordan, 2004; Kim et al., 2005). Yin et al. (Yin et al., 2008) studied the extraction of betulin from *Inonotus Obliquus*. The pretreatment of raw material included drying, pulverizing, and storing. Treatment temperature and flow velocity were fixed at 23℃ and 20ml/min respectively. In research, various experimental conditions of HIPEF method, such as solvent (30-100% (v/v) ethanol, 75% (v/v) methanol, hexane, and acetone), liquid-material ratio (5:1-30:1), electric field intensity (10-70kV/cm), pulse numbers (1-7, pulse width: 2μs) were investigated. The highest extraction yield 6.83±0.10g/kg was obtained by performing the HIPEF extraction at a set of optimal

conditions (solvent: 75% (v/v) ethanol, liquid-material ratio: 25:1, electric field intensity: 40kV/cm, pulse numbers: 2). The conventional extraction method referred to maceration extraction (Zhao et al., 2004) and shows that yield of HIPEF method for 4μs was higher than those with maceration extraction (5.41±0.08g/kg) for 24h under the same experimental conditions. The results indicated HIPEF method could be more efficient to reach a higher extraction yield and reduce time. For extraction non-polar compound such as plant raw material, HIPEF proved itself a promising technique once again.

He et al. (He et al., 2014) extracted chondroitin sulphate from fish bone using HIPEF technique. The extraction treatment was carried out at the flow velocity of 14ml/min and room temperature, with other parameters changed accordingly. Variation of HIPEF parameters (solvent concentration, material-liquid ratio, electric field intensity, and pulse number) and the content of chondroitin sulphate were determined by single factor design experiments. The process conditions were optimized by quadratic general rotary unitized design experiments. To compare HIPEF with conventional extraction methods, control experiments, using the same raw material from the same batch in order to verify the effectiveness of HIPEF, were performed, concluding enzyme method, alkali method, and ultrasonic method (Yang et al., 2011b; Xu et al., 2011; Cheng et al., 2011). The maximum yield of 6.92g/L was achieved in 5min under the extraction conditions as follow: solvent concentration of 3.24% (v/v), liquid-material ratio of 15:1ml/g, electric field intensity of 16.88kV/cm, pulse number of 9 (pulse duration of 18μs). In

comparison with enzyme method (3.43g/L in 4h), alkali method (3.76g/L in 2h), and ultrasonic method (4.87g/L in 23min) were carried under the optimal conditions respectively. HIPEF showed its superiority in the aspect of extraction of chondroitin sulphate, as a fast and efficient method. The purity of extract of chondroitin sulphate was quite high and it did not contain impurity such as glycosaminoglycans.

Yin et al. (Yin et al., 2007) developed a high throughput DNA extraction method from bovine spleen with HIPEF technique. The samples were filtered after smashing and then immediately used for the PEF treatment to isolate DNA from bovine spleens. Two kinds of special solvent A (0.05M ethylenediamine tetraacetic, acid disodium salt (Na_2--EDTA), 0.1M sodium citrate, 2.5% (v/v) polyvinyl pyrrolidon (PVP), 1% (v/v) sodium dodecylsulfate (SDS), NaCl) and B (0.05M Na_2--EDTA, 0.1M sodium citrate, 2.5% (v/v) PVP, 1% (v/v) SDS, NaCl) were prepared for extraction. The effect of NaCl concentration in solvent A and B, heating temperature, diluted time (liquid-material ratio), electric field intensity, and pulse number on extraction process, were discussed in this research. To the other parameters, the flow velocity of the process was adjusted to 2ml/min controlled by pump and pulse width of sawtooth-wave pulses was 2μs. The absorbency value of 260 nm by spectrophotometer (OD_{260}) and absorbency ratio of 260 to 280 nm ($OD_{260/280}$) value were used to express the concentration of genomic DNA in the solution and purity of the extracted genomic DNA respectively. A maximum isolation was achieved when materials suspended in buffer (pH 5.5) was treated at 30kV/cm, 140Hz, pulse

numbers were 8, diluted time was 4, temperature was 65℃, total treatment time was 0.5h, and the NaCl concentration of solvent A and B was 1.6% and 14% (v/v) separately. In comparison with traditional method selected in the literatures, HIPEF performed twice more efficiently than the former in extraction. Eventually this work clearly demonstrated that the HIPEF was effective to reach a high throughput DNA extraction at high-salted, low-acidic conditions.

CONCLUSION AND FUTURE TRENDS

The effects of different factor for HIPEF extraction and optimal parameter for extraction of several kinds of materials had been synthesized and discussed. Based on utility of combination of optimization such as single factor test and orthogonal quadratic design, a set of optimal parameters could be chosen.

In comparison of extraction yield and product content with other extraction methods including conventional and unconventional methods, the results indicated that HIPEF extraction had advantages of higher extract yield, less treatment time, and lower energy consumption. HIPEF extraction works at low temperature and avoids loss of heat-sensitive compounds. In addition, it is relatively easy to realize continuous or batch production adopting HIPEF extraction method, due to its novel design of continuous extraction system. So, HIPEF technique is promising in domain of analytical, pharmaceutical and food industries in the future.

Although the performance of HIPEF technique was good enough, there exist some drawbacks and problems need to be solved in further research.

Exponentially decaying bipolar triangle pulsed waveform with pulse width of 2μs was chosen in this work, which induces energy waste. Utility of square wave pulses with larger width might save a great deal of energy and equipment cost.

The structure of treatment chamber might be improved. At present, the flow rate of the HIPEF system cannot meet the requirement of industrial production. It is largely because the design of the treatment chamber is not reasonable enough. The hole on the insulating plate is so small that the maximum of flow rate is severely limited and the instability of the system cannot be ignored. Some faults such as blocking and short trouble are likely to happen. So, it is necessary to redesign or improve the existing HIPEF system in order to obtain higher efficiency, stability and safety.

All the procedures including pretreatment and post-treatment should be considered better. The process of grinding and stirring as pretreatment is appropriate to enhance extraction and the maximum particle size of material in the liquid which must be kept smaller than the gap of the treatment region in the chamber in order to maintain a proper processing operation. Products with high electrical conductivity reduce the resistance of the chamber and require more energy to

achieve a specific electrical field in consequence. Therefore, when producing high salt products, the salt should be added after processing.

Eventually, the theories describing accurately the mass transfer under HIPEF process are lacking. The complex mechanisms of cell inactivation and substance diffusion are not uncovered well. A deeper understanding of the mechanism of HIPEF and establishment of scientific model of mass transfer contributes to further research.

ACKNOWLEDGEMENT

This work is financially supported by the Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20100181120076), Project funded by China Postdoctoral Science Foundation (No. 2013M530400, 2014T70871) and the National Natural Science Foundation of China (No. 21376150).

REFERENCES

Abah, S. E., and Egwari, L. O. (2011). Methods of extraction and antimicrobial susceptibility testing of plant extracts. *Afr. J. Basic Appl. Sci.* **3(5)**: 205-209.

Anthea, M., Hopkins, J., McLaughlin, C. W., Johnson, S., Warner, M. Q., LaHart, D., and Wright, J. D. (1993). Human Biology and Health. Prentice Hall, Englewood Cliffs, NJ.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., and Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.* **117(4)**: 426-436.

Barbosa-Cánovas, G. V., Gongora-Nieto, M. M., Pothakamury, U. R., and Swanson, B. G. (1999). Preservation of Foods with Pulsed Electric Fields. Academic Press Ltd, London.

Boulaaba, A., Kiessling, M., Töpfl, S., Heinz, V., and Klein, G. (2014). Effect of pulsed electric fields on microbial inactivation and gelling properties of porcine blood plasma. *Innov. Food Sci. Emerg.* **23**: 87-93.

Brereton, K. R., and Green, D. B. (2012). Isolation of saccharides in dairy and soy products by solid-phase extraction coupled with analysis by ligand-exchange chromatography. *Talanta*. **100**: 384-390.

Castro, A. J., Barbosa-Cánovas, G. V., and Swanson, B. G. (1993). Microbial inactivation of foods by pulsed electric fields. *J. Food Process Pres*. **17**:47-73.

Chan, C. H., Yusoff, R., Ngoh, G. C., and Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *J. Chromatogr. A.* **1218**: 6213-6225.

Cheng, C. Y., Duan, Z. H., Fan, J., Zhou, K. G., and Chang, H. (2011). Optimization of chondroitin sulfate extraction from tilapia byproduct with response surface methodology. *Food Sci. Technol*. **36**: 128-133.

Donsi, F., Ferrari, G., and Pataro, G. (2010). Applictions of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *Food Eng. Rev*. **2(2)**: 109-130.

Eduardo, P., and Iñigo, M. D. M. (2015). Olive oil pilot-production assisted by pulsed electric field: Impact on extraction yield, chemical parameters and sensory properties. *Food Chem.* **167**: 497-502.

El-belghiti, K., and Vorobiev, E. (2005). Modelling of Solute Aqueous Extraction from Carrots subjected to a Pulsed Electric Field Pre-treatment. *Biosyst. Eng.* **90(3)**: 289-294.

Ganeva, V., and Galutzov, B. (1999). Electropulsation as an alternative method for protein extraction from yeast. *FEMS Microbiol. Lett.* **174**: 279-284.

Cardenas-Toro, F. P., Forster-Carneiro, T., Rostagno, M. A., Petenate, A. J., Filho, A. M., and Meireles, M. A. A. (2014). Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber. *J. Supercrit. Fuild*. **93**: 42-48.

Glade, M. J. (1996). Calcium and related nutrients - A workshop sponsored by the panel on calcium and related nutrients of the standing committee on the scientific evaluation of dietary reference intakes, Food and Nutrition Board, Institute of Medicine, Washington, DC, - July 9-10, 1996. *Nutrition.* **13(7-8)**: 721-724.

Goettel, M., Eing, C., and Gusbeth, C. (2013). Pulsed electric field assisted extraction of intracellular valuables from microalgae. *Algal. Res.* **2(4)**: 401-408.

He, G. D., Yin, Y. G., Yan, X. X., and Yu, Q. Y. (2014). Optimisation extraction of chondroitin sulfate from fish bone by high intensity pulsed electric fields. *Food Chem.* **164**: 205-210.

Ho, S. Y., Mittal, G. S., Cross, J. D., and Griffiths, M. W. (1995). Inactivation of Pseudomonas fluorescens by high voltage electric pulses. *J. Food Sci.* **60(6)**: 1337-1343.

Janositz, A., and Knorr, D. (2010). Microscopic visualization of pulsed electric field induced changes on plant cellular level. *Innov. Food Sci. Emerg.* **11**: 592-597.

Jarrett, O. O., Ogunbosi, B. O., and Ayoola, O. O. (2013). Paediatric endocrine disorders at the University College Hospital, Ibadan: 2002--2009. *Ann. Ibadan Postgrad. Med.* **11**: 96-101.

Jeyamkondan, S., Jayas, D. S., and Holley, R. A. (1999). Pulsed electric field processing of foods: A review. *J. Food Protect.* **62(9)**: 1088-1096.

Jin, Y., Wang, M., Lin, S. Y., Guo, Y., Liu, J. B., and Yin, Y. G. (2011). Optimization of extraction parameters for trehalose from beer waste brewing yeast treated by high-intensity pulsed electric fields (PEF). *Afr. J. Biotechnol*. **10(82)**: 19144-19152.

Jordan, K. Z. (2004). Biologically active compounds from *Aphyllophorales* (Polypore) fungi. *J. Nat. Prod*. **67(2)**: 300-310.

Kim, Y. O., Han, S. B., Lee, H. W., Ahn, H. J., Yoon, Y. D., Jung, J. K., Kim, H. M., and Shin, C. S. (2005). Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of Inonotus obliquus. *Life Sci*. **77**: 2438−2456.

Kinosita, K. J., and Tsong, T. Y. (1977). Voltage induced pore formation and haemolysis erythrocytes. *Biochim. Biophys. Acta*. **471**: 227-242.

Kvasnica, M., Sarek, J., Klinotova, E., Dzubak, P., and Hajduch, M. (2005). Synthesis of phthalates of betulinic acid and betulin with cytotoxic activity. *Bioorgan. Med. Chem*. **13**: 3447-3454.

Li, C. T., Mao, X. X., and Xu, B. J. (2012). Pulsed Electric Field Extraction Enhanced Anti-coagulant Effect of Fungal Polysaccharide from Jew's Ear (*Auricularia auricula*). *Phytochem. Analysis*. **24(1)**: 36-40.

Lin, S. Y., Wang, L. Y., Jones, G., Trang, H., Yin, Y. G., and Liu, J. B. (2012). Optimized extraction of calcium malate from eggshell treated by PEF and an absorption assessment in vitro. *Int. J. Biol. Macromol*. **50**: 1327-1333.

Liu, M. Y., Zhang, M. S., Lin, S. Y., Liu, J. B., Yang, Y., and Jin, Y. (2012). Optimization of extraction parameters for protein from beer waste brewing yeast treated by pulsed electric fields (PEF). *Afr. J. Microbiol. Res*. **6(22)**: 4739-4746.

Mousdale, D. M. and Coggins, J. R. (1991). Amino acid synthesis. **In:** Target Sites for Herbicide Action, pp. 29-39. Kirkwood, R. C., Eds., Plenum Press, London.

Oh, S. H., Choi, J. E., and Lim, S. C. (2006). Protection of betulin against cadmium-induced apoptosis in hepatoma cells. *Toxicology*. **220**: 1-12.

Parniakov, O., Lebovka, N. I., Van-Hecke, E., and Vorobiev, E. (2014). Field Assisted Pressure Extraction and Solvent Extraction from Mushroom (*Agaricus Bisporus*). *Food Bioprocess Tech.* **7**: 174-183.

Pourzaki, A., Mirzaee, H., and Kakhki, A. H. (2013). Using pulsed electric field for improvement of components extraction of saffron (*Crocus Sativus*) stigma and its pomace. *J. Food Process Pres.* **37(5)**: 1008-1013.

Prentice, A. (2013). Nutritional rickets around the world. *J. Steroid Biochem*. **136**: 201-206.

Puértolas, E., López, N., Saldaña, G., Álvarez, I., and Raso, J. (2010). Evaluation of phenolic extraction during fermentation of red grapes treated by a continuous pulsed electric fields process at pilot-plant scale. *J. Food Eng*. **98(1)**: 120-125.

Qin, B. L., Zhang, Q., Barbosa-Cánovas, G. V., Swanson, B. G., and Pedrow, P. D. (1994). Inactivation of microorganisms by pulsed electric fields with different voltage waveforms. *IEEE Trans. Dielec. Insul.* **1(6)**: 1047-1057.

Ross, A. C., Man, J. E., Abrams, S.A., Aloia, J. F., Brannon, P. M., Clinton, S. K., Durazo-Arvizu, R. A., Gallagher, J. C., Gallo, R. L., Jones, G., Kovacs, C. S., Mayne, S. T., Rosen, C. J., and Shaoses, S. A. (2011). The 2011 dietary reference intakes for calcium and vitamin D: What dietetics practitioners need to know. *J. Am. Diet. Assoc.* **111(4)**: 524-527.

Sale, A. J. H., and Hamilton, W. A. (1967). Effects of high electric fields on microorganisms I. Killing of bacteria and yeast. *Biochimt. Biophys. Acta.* **148**: 781-788.

Schoenbach, K. H., Peterkin, F. E., Alden, R. W., and Beebe, S. J. (1997). The effect of pulsed electric fields on biological cells: Experiments and applications. *IEEE Trans. Plasma Sci.* **25(2)**: 284-292.

Smith, R. M. (2003). Before the injection-modern methods of sample preparation for separation techniques. *J. Chromatogr. A.* **1000(1-2)**: 3-27.

Sun, W. F., Zhou, S. M., and Wang, Q. (2007). Research progress of integrated utilization of spent brewer's yeast. *Chem. Eng. Prog*. **27**: 990-1000.

Tang, Y., and Li, H. (2002). Effect of fermentation by lactobacillus on nutrition and physiochemical property of super-micro-smashing hog-bone powder. *T. Chinese Soc. Agr. Eng*. **18**: 118-122.

Tsong, T. Y. (1990). Electrical modulation of membrane proteins: Enforced conformational oscillations and biological energy signals. *Annu. Rev. Biophys. Chem*. **19**: 83-106.

Wang, L. J., and Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci. Tech.* **17(6)**: 300-312.

Wu, L., Zhao, W., Yang, R. J., and Chen, X. C. (2014). Effects of pulsed electric fields processing on stability of egg white proteins. *J. Food Eng.* **139**: 13-18.

Wurdemann, H. A., Aminzadeh, V., Dai, J. S., Reed, J., and Purnell, G. (2011). Category-based food ordering processes. *Trends Food Sci. Tech.* **22(1)**: 14-20.

Xi, J. (2013). High-pressure processing as emergent technique for the extraction of bioactive ingredients from plant materials. *Crit. Rev. Food Sci*. **53(8)**: 837-852.

Xu, R. C., Sun, A. D., Zhang, D. Q., Rao, W. L., and Wang, Y. X. (2011). Optimization of the extraction parameters of chondroitin sulfate from sheep cartilage through quadratic regression orthogonal design. *Food Sci. Technol.* **36**: 128--132.

Yan, H. S., Zhang, S. Q., Luo, X. P., Hou, L. L., and Liang, Q. (2008). HHPE: Improving the stability and antioxidant activity of bioactive components from *Hedyotis diffusa* willd. *J. Biotechnol.* **136(1)**: 468--469.

Yang, B., Jiang, Y. M., and Shi, J. (2011a). Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit -- A review. *Food Res. Int.* **44(7)**: 1837-1842.

Yang, S. G., and Ning, H. (2011b). Extraction of chondroitin sulfate from skate cartilage. *J. East. Liaoning Univ.* **18**: 104-107.

Yin, Y. G., Zhao, W. Q., Guan, W., and Jiang, C. S. (2002). Experimental study on a food pasteurization system by using a high voltage pulsed field. *Agr. Eng. Trans.* **33**: 67-69.

Yin, Y. G., Han, Y. Z., and Yong, H. (2006). Pulsed electric field extraction of polysaccharide from *Rana temporaria chensinensis* David. *Int. J. Pharm*. **312**: 33-36.

Yin, Y. G., Jin, Z. X., Wang, C. L., and An, W. Z. (2007). The effect of pulsed electric field on DNA extraction from bovine spleens. *Sep. Purif. Technol*. **56**: 127-132.

Yin, Y. G., and He, G. D. (2008a). A fast high-intensity pulsed electric fields (PEF)-assisted extraction of dissoluble calcium from bone. *Sep. Purif. Technol*. **61**: 148-152.

Yin, Y. G., Cui, Y. R., and Ding, H. W. (2008b). Optimization of betulin extraction process from Inonotus Obliquus with pulsed electric fields. *Innov. Food Sci. Emerg*. **9**: 306-310.

Zbinden, M. D. A., Sturm, B. S. M., Nord, R. D., Carey, W. J., Moore, D., Shinogle, H., and Stagg-Williams, S. M. (2013). Pulsed electric field (PEF) as an intensification pretreatment for greener solvent lipid extraction from microalgae. *Biotechnol. Bioeng*. **110(6)**: 1605-1615.

Zhang, Y., Li, H. F., Ma, Y., Jin, Y., Kong, G. H., and Lin, J. M. (2014). Microwave assisted extraction -- solid phase extraction for high-efficient and rapid analysis of mono-saccharides in plants. *Talanta*. **129**: 404-410.

Zhao, F. Q., Pu, H. S., and Han, C. J. (2004). Studies on anti-mutantion active constituents of the Fuscoporia oblique. *J. Med. Sci. Yanbian Univ*. **27**: 250-252.

Zhao, W., Yang, R. J., Tang, Y. L., Zhang, W. B., and Hua, X. (2009). Investigation of the protein -- protein aggregation of egg white protein under pulsed electric fields. *Agric. Food Chem.* **57**: 3571-3577.

Zhao, W. Z., Yu, Z. P., Liu, J. B., Yu, Y. D., Yin, Y. G., Lin, S. Y., and Chen, F. (2011). Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design. *J. Sci. Food Agr.* **91(12)**: 2201-2209.

Table 1 List of optimal conditions of HIPEF extraction of bioactive compounds.

	Optimal parameters						
Bioactive compounds	Electric field intensity[kV/cm]	Pulse number	Ratio of solvent to raw material[ml/g]	Solvent	Environment (temperature, pH)	flow velocity [ml/min]	Reference
Calcium malate from eggshell	20	12	50:1	6.0% malic acid	RT	25	Lin et al., 2012
Dissoluble calcium from bone	70	12	40:1	1.25% citric acid	RT	11.775	Yin et al, 2008a
DNA from bovine spleens	30	8	4:1	special solvent	65℃, pH = 5.5	2	Yin et al., 2007
Protein from beer waste	10	8	40:1	water	RT	24	Liu et al., 2012

brewing yeast							
Trehalose from beer waste brewing yeast	19.97	6	30: 1	water	RT, pH = 7	24	Jin et al., 2011
Polysaccharides from corn silk	30	3	50: 1	water	RT	25	Zhao et al., 2011
Polysaccharides from *Rana temporaria chensinensis* David	20	3	Not mentioned	0.5% KOH	RT	26	Yin et al., 2006
Polysaccharides from Jew's Ear	24	6	30: 1	water	below 40℃,pH = 8	5	Li et al, 2012
Betulin from	40	2	25:	75%	23	20	Yin et

Inonotus Obliquus			1	ethanol			al., 2008b
Chondroitin sulfate from fish bone	16.88	9	15: 1	3.24% NaOH	RT	14	He et al., 2014

RT: room temperature

Table 2 List of comparison between HIPEF and other extraction methods, the conditions of all methods have been optimized.

		Operating conditions				
Bioactive compounds	Extraction methods	Solvent(solvent: raw material, ml/g)	Temperature (°C)	Total treatment time	Extraction yield (%) or product content (mg/L)	Reference
Dissoluble calcium from bone	HIPEF	1.25% citric acid(40:1)	RT	8.5min	4324.8mg/L	Yin et al., 2008a
	Blank	water(40:1)	RT	24h	22.032mg/L	
	Stilness	citric acid(40:1)	RT	24h	210.522mg/L	
	Boiling	citric acid(40:1)	100	40min	506.981mg/L	
	Microwave	citric acid(40:1)	not mentione	15min	1413.72mg/L	

			d			
Trehalose from beer waste brewing yeast	HIPEF	water(30:1)	RT	6min	2.635%	Jin et al., 2011
	UAE	water(40:1)	RT to 100	380min	7.72%	
	MAE	water(30:1)	80 to 100	213min	9.24%	
Polysaccharides from corn silk	HIPEF	water(50:1)	RT	4min	7.31%±0.15%	Zhao et al., 2011
	Boiling	water(60:1)	100	60min	5.46%	
	MAE	water(50:1)	RT to 100	33min	6.18%	
Polysaccharides from *Rana temporaria chensinensis* David	HIPEF	0.5% KOH	RT	a few minutes	55.59% and 43.15mg/L	Yin et al., 2006
	Alkali	5%KOH	45	2h	19.78% and 10.78mg/L	
	Enzyme	pepsin	50	5h	24.44% and 14.93mg/L	

	Comound	1%KOH+enzymes	50	6h	31.38% and 16.81mg/L	
Betulin from *Inonotus Obliquus*	HIPEF	75% ethanol(25:1)	23	1.25min	(6.83±0.10)g/kg	Yin et al., 2008b
	Maceration	75% ethanol(25:1)	24	24h	(5.41±0.08)g/kg	
Chondroitin sulfate from fish bone	HIPEF	3.24% NaOH(15:1)	RT	5min	6.92g/L	He et al., 2014
	Enzyme	pancreatic enzyme	50	4h	3.43g/L	Yang et al., 2011
	Alkali	7% NaOH(6.5:1)	35	2h	3.76g/L	Xu et al., 2011
	Ultrasonic	2.4% NaOH(7.9:1)	RT	23min	4.87g/L	Cheng et al., 2011

RT: room temperature

Table 3 Comparison of extraction rate of three continuous extraction methods (HIPEF, UAE, MAE)

Bioactive compounds	Extraction methods	Extraction rate (μg/s)
Trehalose from beer waste brewing yeast	HIPEF	103.15
	UAE	3.03
	MAE	6.46

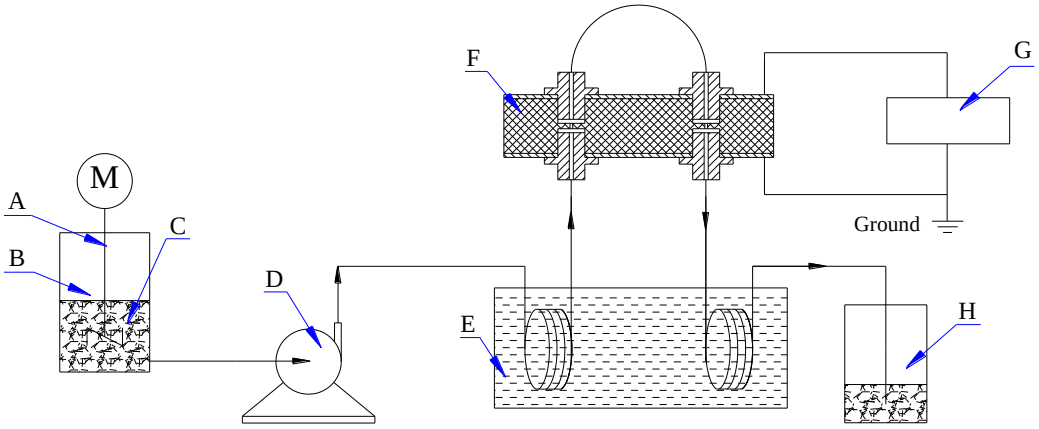

Figure 1 Schematic of HIPEF treatment system (A: agitator, B: material tank, C: mixture of material and solvent, D: peristaltic pump, E: thermostatic water bath, F: HIPEF treatment chamber, G: PEF generator, H: product tank)

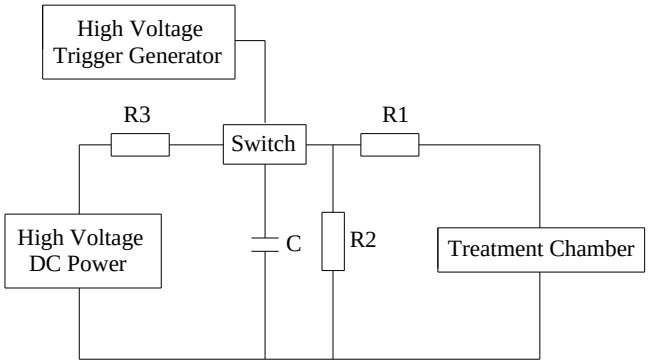

Figure 2 Schematic of high-intensity pulsed electric fields circuit (Yin et al., 2008a)

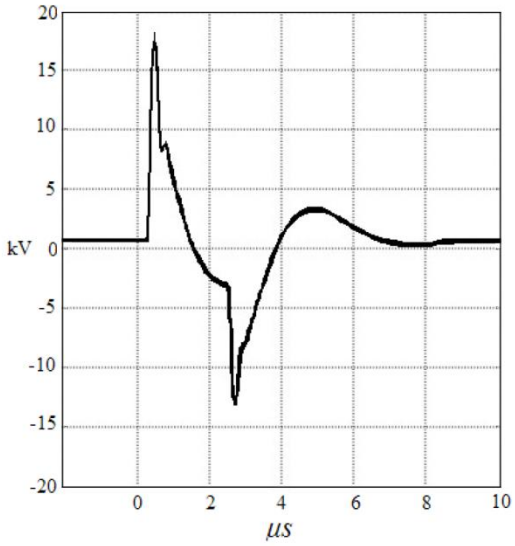

Figure 3 Form of pulsed wave (Jin et al., 2011)

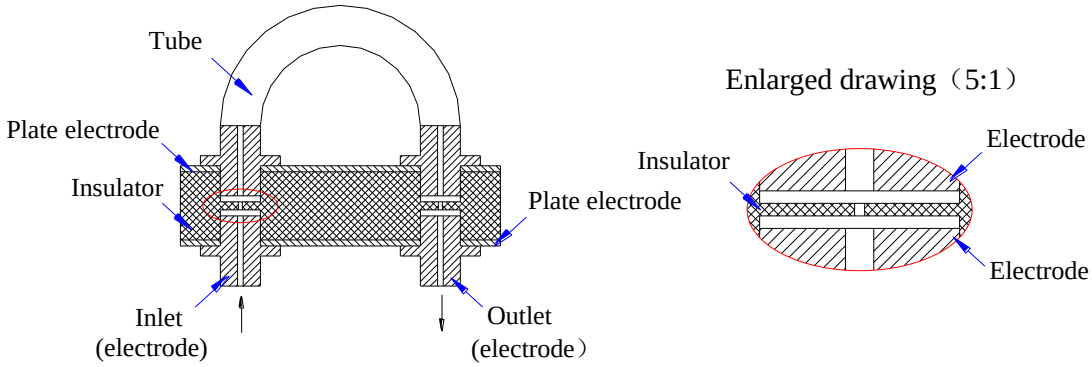

Figure 4 Schematic of HIPEF treatment chamber

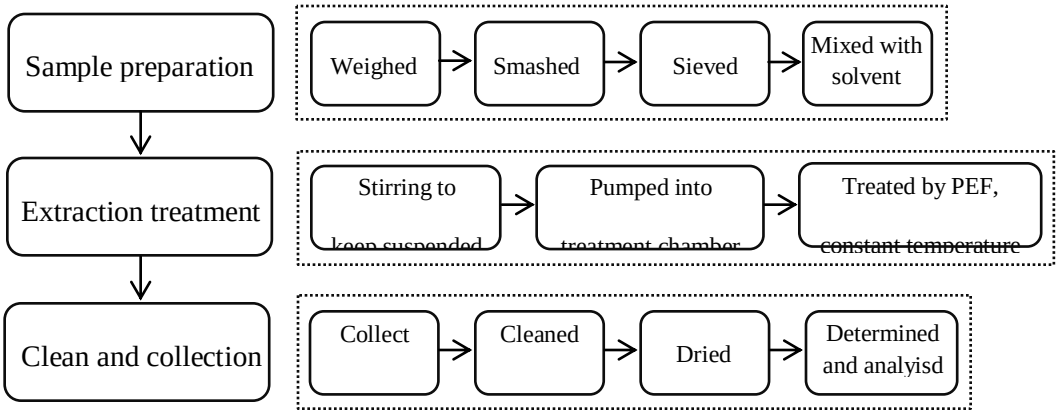

Figure 5 Schematic procedures of HIPEF processing

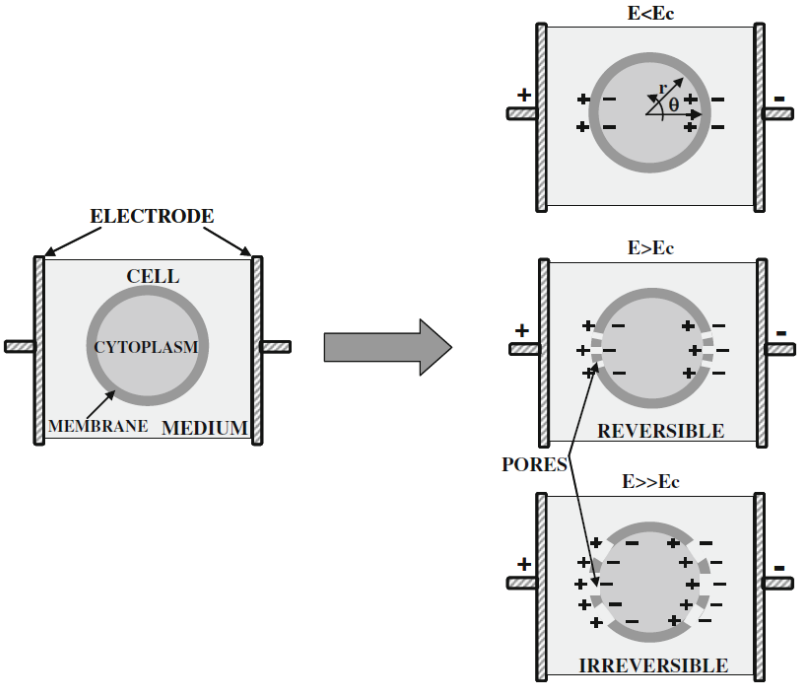

Figure 6 Biological cell in an electric field E. Electroporated area is represented with a dashed line (Ec: critical electric field intensity) (Donsi et al., 2010)