ABSTRACT

Due to their established health benefits, phytochemicals such as phenolics, carotenoids, sterols, and alkaloids are becoming more and more popular. However, some of these phytochemicals may not be amenable to typical extraction techniques. Examples of innovative methods include supercritical fluid extraction (SFE), microwave extraction (MWE), pulsed electric field (PEF), high-pressure processing (HPP), and ultrasonic extraction (UE), and ohmic heating (OH). Supercritical fluid extraction is one of the methods that is most often researched and has already found commercial use for number of products. The most used supercritical fluid for agricultural products is carbon dioxide. Additionally growing, and already having commercial uses, is microwave extraction. By using electromagnetic radiation to quickly and uniformly heat the matrix, this technique produces extractions with high quantities of phytochemicals and few impurities. Despite current interest in pulsed electric fields, which have several benefits over thermal treatments, their usage is still limited to a small number of industrial units. Ohmic heating occurs when alternating current is sent through the matrix, heat is produced by sample's electrical resistance discharging. Low-frequency electrical energy is demonstrated to promote the extraction of phytochemicals since it dissipates into heat quickly and uniformly. High-pressure processing has potential to improve diffusion, increase mass transfer and cell permeability, and maintain food quality. HPP reduces negative effects of heat while greatly enhancing texture. HPP employs high pressures and moderate temperatures in comparison to traditional extraction techniques. With the employment of a combination of cutting-edge technologies that will enable the production of high-quality phytochemicals with little degradation, future of phytochemical extraction is undoubtedly bright.

Keywords: Supercritical fluid extraction, Pulsed electric field, High-pressure processing, Ultrasonic extraction and Ohmic heating.

INTRODUCTION

Plants serve as the most diverse natural component, and traditional habits mainly depend on them for their therapeutic properties. The products obtained from the natural substances act as a source for preparing foodstuffs, herbal medicines, and beauty products (Shinde VM and Dhalwal K, 2008). A variety of chemical substances found in plants can be utilised to treat both chronic and infectious disorders (Duraipandiyan et al., 2006). There are several different compounds that make up phytochemicals, including carotenoids, phenolics, alkaloids, and sterols (Shinde et al., 2008). They can be found in fruits, seeds, vegetables, nuts, and plants and can be obtained usually by various extraction methods with the help of solvents like chloroform, methanol, and ethyl acetate (Marleny et al., 2010) Extraction is the crucial step in the process of discovering novel drugs and developing them from plants (Shinde et al., 2008). In general, the heat applied for the removal of solvents may not be appropriate for thermolabile substances, and also moreover, legislative guidelines for using organic solvents are getting stringent. Hence, the traditional process of extraction which includes separating the active ingredient from the raw material by employing a suitable solvent is now changing significantly into modern techniques (Shinde and Dhalwal 2009). Due to their capacity to resolve many of the drawbacks of traditional technologies, the developing technologies including SFE, PEF, MWE, UE, and HPP are becoming more widely used (Marleny et al., 2010, Zhao et al., 2014, Devgun et al., 2009, Noelia Lopezet al., 2009, Leksawasdi et al., 2022). Innovative extraction techniques, as opposed to traditional options, provide larger yields in very little time, need fewer organic solvents, and use less energy (Danijela Bursac Kovacevic et al., 2018).
The SFE is a process that separates or extracts chemical components from a matrix by employing carbon dioxide as the solvent for extraction under conditions of critical temperature and pressure (Zhao et al., 2014). The phase separation can enable the accumulation of pure CO2 solvent (gas), allowing it to be redistributed into storage and available for reuse, lowering total energy expenditures and boosting overall sustainability.

The pulsed electric field uses a high electric field and transmits shorter pulses at room temperature when the product is placed between two electrodes. This technique has shown increased yield for the extraction when an electric strength of less than 10kv/cm is given. This technique enhances the cell membrane permeability and also extraction efficiency (Bazhal et al., 2001).

One of the modern, cutting-edge extraction methods being researched at the moment is microwave assistance. In this technique, both solvents and microwaves are used to extract the plant metabolites. Due to its stability and simplicity in usage, this method has shown to be healthy for a large number of specimens (Iqra Akhtar et al., 2019).

In ultrasonic extraction, greater ultrasonic waves are used to generate compression and expansion cycles which leads to cavitation, cell membrane rupturing, and then causes solvent infiltration into the cells (Tang et al., 2021). When compared to traditional methods of extraction, UE is advantageous since it is time-saving, simple, and eco-friendly as well as it produces high-quality extracts at a reasonable cost (Crupi et al., 2018). High pressure processing is a method used for processing of foods to maintain its quality by use of higher pressure. It has recently started developing in the extraction process of bioactive components. This technique has several advantages over other extraction techniques as it gives yields of greater extraction, the extraction solution contains very less impurities, makes use of little heat which prevents the thermal degradation of the compound (Corrales et al., 2008). In this present study, the various innovative technologies emerging for the extraction of phytoconstituents from natural sources will be explained in detail.

**Supercritical Fluid Extraction:** Since the late 1800s, supercritical fluid technology has been used to extract various chemical compounds. CO2 is used as a mobile phase and the entire chromatographic flow path is driven under pressure. When a fluid’s pressure and temperature are higher than their respective critical pressure and critical temperature, the fluid is said to be supercritical. No phase transition occurs above the critical temperature, meaning that in spite of how much pressure is applied, the fluid cannot change from a gaseous state to a liquid state. There is just one phase in the supercritical region, which is described as neither a gas nor a liquid and possesses physical and thermal characteristics halfway between a pure gas and a pure liquid (Gopaliya et al., 2014). SFE is carried out by dissolving the desired plant sample component under strictly monitored pressure and temperature. The extracted component is then separated from the supercritical fluid by a substantial reduction in solution pressure. The extract obtained using this technique will not contain any organic residues and is hence most widely used in the pharmaceutical and food industries. The constituents such as alkaloids, glycosides, essential oils, and lipids can be extracted easily and economically in an eco-friendly manner (Sihvonen et al., 1999) as the mobile phase(CO2) being used is non-toxic, cheap, and stable (Gongb et al., 2015). This can be an advantage for the compounds that are thermally labile as the critical temperature of CO2 is low (Tozzi et al., 2008). This technique is preferable to conventional extraction methods as the supercritical fluids may easily flow through substances and have greater transit capabilities than traditional organic solvents, which increases the extraction efficiency and yield of the target molecules (Samar Al Jitan S et al., 2018). It also allows thorough extraction since the new fluid is continually compelled to pass through the samples (Bubalo et al., 2018). The disadvantage associated with this technique is it cannot be used for the polar solutes as CO2 is non-polar in nature and also have less dissolving capacity although which can be improved by using solvent modifiers.

**Table 1: Compounds extracted from plant sources using SFE technology.**

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Extracted compounds</th>
<th>Pressure</th>
<th>Temperature (OC)</th>
<th>Time (min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ oil</td>
<td>Limonene, 2-methyl-2-buten, hexanal, linoleic acid, 2,4-heptadienal</td>
<td>20</td>
<td>40</td>
<td>-</td>
<td>(Piras et al., 2009)</td>
</tr>
<tr>
<td>Baccharis dracunculifolia (Leaves)</td>
<td>Limonene, nerolidol, phenolics, artepillin</td>
<td>400</td>
<td>60</td>
<td>20</td>
<td>(Piantino et al., 2008)</td>
</tr>
<tr>
<td>Carica papaya (Seeds)</td>
<td>Papain, carpaine, benzyl isothiocyanate, pseudocarpaine, chymopapain.</td>
<td>100</td>
<td>40</td>
<td>20</td>
<td>(Barroso et al., 2014)</td>
</tr>
</tbody>
</table>
**Pulsed Electric Field (PEF):**

It is a non-thermal technique in which the sample is positioned between two conductive electrodes and subjected to extremely brief, high-voltage pulses that are transmitted through the sample (Thulasidas et al., 2019). The outcome is an increase in the conductivity, porosity, and permeability of the sample's cell membrane. This makes it easier for substances bound inside the cell membrane to diffuse out into the surrounding solution, allowing them to be extracted. Cells subjected to an electric field segregate molecules as per their charge as the molecules in the cell membrane are of dipole nature. The electric field causes the temporary disruption of the proteins and lipid bilayer of the cell membrane. This technique often works best for liquid substances as the electric field passes through easily. It is a non-thermal technique, helping in retaining the chemical characteristics of the molecule by preventing the negative effects of heating (Martinez et al., 2020). For sensitive plant tissues, an electric field between 0.1 and 10 kV/cm is sufficient; nevertheless, strong materials, including seeds, require high intensities (between 10 and 20 kV/cm) for effective extraction (Thulasidas et al., 2019). It is an effective extraction technology when compared to other traditional extraction methods in terms of time, energy, cost, and also being environmentally conscious (Thulasidas et al., 2019).

According to research on *Humulus lupulus* pellets, the application of pulsed electric field has enhanced α acids and β acids extraction from 21-100% and 9-120% respectively and also increased the extraction of sesquiterpenes by 1.3 times (Ntourtoglou et al., 2020). In case of extraction of intracellular components from microalgae, a pre-treatment of the sample should be performed to enhance the extraction (Parniakov et al., 2015). When compared to traditional extraction, PEF greatly increased the extraction of proteins, polysaccharides, and polyphenols from white button mushrooms (Xue and Farid, 2015).

**Microwave Extraction (MWE):**

The use of MAE as a method to extract bioactive chemicals from a range of plants and natural remnants has caught the interest of researchers (Anokwuru et al., 2011). Microwaves emit electromagnetic radiation with frequencies ranging from 300MHz to 300GHz and wavelength ranging from 1 cm to 1 m. The EM waves include both magnetic and electric fields in a perpendicular direction. The microwaves were initially used to heat up materials that could partially absorb electromagnetic energy and transform it into heat. In addition to heating organic molecules, microwaves also generate dipole rotation, which breaks down hydrogen bonds and leads to improve the solvents' ability to penetrate the plant matrix (Hudaib et al., 2003). The advantages of this technique over other traditional methods include less extraction time which takes few seconds to minutes for completion, pesticide remnants and heavy metals can be detected even in trace amounts, and also it makes use of agitation during extraction which allows mass transfer (Mandal et al., 2007). MAE is used to detect heavy metals and other contaminants found in soil samples. There is a number of studies on the extraction of glycosides (Javad et al., 2014), essential oils (Wang et al., 2010), flavonoids (Xiao et al., 2008), phenolics (Gallo et al., 2010), and other substances. The main drawback of this method includes inconsistent heating and/or overheating of the extract due to variable dielectric characteristics of the solvent and plant material, which may limit extraction efficiency or induce thermal degradation of phenolic acids (Samar et al., 2018).

### Table 2: Conditions for extraction from plant sources using PEF technique.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Frequency, intensity, and no. of pulses employed</th>
<th>Solvent used</th>
<th>Yield</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminalia catappa</td>
<td>1Hz, 0.75kV/cm, and 50 pulses</td>
<td>water</td>
<td>74.6%</td>
<td>Moghaddam et al., 2020</td>
</tr>
<tr>
<td>Prunus avium</td>
<td>5Hz, 0.5-1kV/cm</td>
<td>Acidified ethanol</td>
<td>40%</td>
<td>Pataro et al., 2017</td>
</tr>
<tr>
<td>Nepeta binaludensis</td>
<td>1Hz, 6kV, 60 pulses</td>
<td>Ethanol</td>
<td>11.36%</td>
<td>Mahalleh et al., 2019</td>
</tr>
</tbody>
</table>

### Table 3: Conditions for extraction from plant sources using MWE.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Solvent used(ml)</th>
<th>Time(sec)</th>
<th>Power(watt)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitis vinifera</td>
<td>50% ethanol (50)</td>
<td>3600</td>
<td>200</td>
<td>(Christina Drosou et al., 2015)</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>60% Acetone (20)</td>
<td>60</td>
<td>750</td>
<td>Charalampos Proestos and Michael Komaitis 2008</td>
</tr>
<tr>
<td>Achillea millefolium</td>
<td>70% ethanol (40)</td>
<td>33</td>
<td>170</td>
<td>Milica Milutinovicet al., 2015</td>
</tr>
</tbody>
</table>
High-Pressure Processing (HPP):
High pressure is used to trigger a variety of processes such as the conversion from one phase to the other, a modification in molecular structure, etc. that cause a reaction that promotes a reduction in volume, hence improving extraction efficiency (Xi et al., 2011). The pressure would damage both the internal and external cell structures resulting in minimizing the resistance to mass transfer within the cell. As a response, the bioactive material will be collected abandoning the other components like flavonoids, alkaloids, pigments, and saponins (Hall and J.E. 2015, Linton et al., 2001). Pressure is crucial in this technique since it directly correlates with the solubility of component (Sanchez-Moreno et al., 2004). The output of bioactive substances is elevated with a rise in pressure. The pressure employed is around 100 to 1,000 Mega Pascal's (MPa), whereas the greatest pressures achieved in high-pressure supercritical extraction are around 100 MPa and 10 MPa, respectively (Ziqiang et al., 2000). The pressure inside the cell is lower when compared to the outside which creates a gradient of higher pressure under which solvents penetrate quickly through tissues and into the interior of the cells. In contrast to supercritical and conventional fluid extraction technologies, HPP accomplishes extraction more quickly. HPE has recently been employed as an effective and rapid way of determining bioactive chemicals since it was shown to be a quicker and more productive form of extraction than standard extraction procedures (Khan et al., 2018).

Comparison of yield extracted from Carica papaya using different techniques
1. Traditional extraction - 0.1MPa for 30minutes - 3.94%
2. Ultrasonic extraction - for 5minutes - 4.19%, for 15minutes - 4.75%
3. High Pressure Processing - 500 MPa for 5 minutes - 5.39%, for 15minutes - 6.49%.

Ohmic Heating (OH)
Ohm’s law is crucial to understand ohmic heating. Ohmic extraction refers to any essential oil extraction procedure that uses electrical currents (without pulsations) to produce volumetric thermal energy. Present extraction process involves both thermal and non-thermal mechanisms (Mohsen Gavahian et al., 2019). Researchers found that the process of extraction temperature impacts the quality of extracts (Kumari et al., 2016). An alternative for hydrodistillation is ohmic-assisted hydrodistillation (OAHD) and alternative for steam distillation is ohmic-accelerated steam distillation (OASD). Both the distillation techniques were useful in extraction of essential oils. (Gavahian and Farahnaky 2018).
When compared to traditional heating, ohmic heating advantages includes less process time, reduced energy consumption, low environmental impacts, producing high quality of essential oils and improved extraction yield. Disadvantages includes capital investment, electrode corrosion at higher pH and advanced engineering calculations. (Mohsen Gavahian et al., 2019).

CONCLUSION

The rising market for health care and food commodities obtained from natural sources has compelled enterprises to develop suitable and effective extraction techniques that retain the positive effects of active constituents. The above-discussed techniques are among such innovative methods which occur only for a few seconds to minutes. These techniques not only produce good yield but also retain their authenticity and activity due to low heat application. Also, in comparison to traditional processes of extraction, these methods are safe, environment-friendly, and consume less solvent which automatically decreases the cost. A lot of research has been done on these methods and hence to meet the need for high-quality products on market, the process must be amplified.

REFERENCES


12. Dranca, F. and M. Oroian, 2016. Optimization of ultrasound-assisted extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC) from eggplant (Solanum melongena L.) peel. Ultrasonics Sonochemistry., 31: 637-646.

13. Drosov, C., K. Kyriakopoulou, A. Bimpilas, D. Tsimogiannis and M. Krokida. 2015. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification by products. Industrial Crops and Products., 75: 141-149.


34. Martinez, JM., 2020. Pulsed electric field assisted extraction of valuable compounds from microorganisms. Comprehensive Reviews in Food Science and Food Safety., 19: 530-552.


39. Parniakov, O., 2015. Pulsed electric field and pH assisted selective extraction of intracellular components


43. **Proestos, C. and M. Komaitis**, 2008. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT - Food Science and Technology.*, 41: 652-659.


