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Conference Paper

Ultrasound-assisted Extraction as a Potential Method to Enhanced Extraction of Bioactive Compound

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ABSTRACT

Ultrasound-assisted Extraction (UAE) is one of the potential ways to increase the yield and quality of bioactive compounds. Besides, the UAE is environmentally friendly since the solvent used is a lesser, non-toxic solvent, time-saving, and operated at a lower temperature, leading to the increase of mass transfer. Ultrasound is one of the wave sounds that have a frequency range that is above the range that can be heard by humans (20 MHz). This wave could cause the breakdown in plant tissue via cavitation, the forming of vacuum bubbles from a wave sound on a liquid material. The breakdown of plant tissue leads to the release of bioactive compounds. These compounds, then, are dissolved in the solvent. The types of bioactive compounds that can be extracted using ultrasound waves are polyphenols, carotenoids, aromatic compounds, and polysaccharides from plant matrix. The UAE method is affected by the temperature, frequency, and amplitude of the wave, density, types of solvent, extraction time, and plant matrix. This article would review the principles of UAE method, cavitation, the characteristic of tissue exposed to ultrasound, and the devices used during the extraction.

Keywords: Bioactive, cavitation, extraction, ultrasound

Introduction

Ultrasound treatment gained tremendous consideration as an effective food extraction method or well-known as ultrasound-assisted extraction (UAE). High soundwave frequency can break low-energy chemical bounding of plant tissue by the formation of cavitation, thereby allowing the extraction of bioactive compounds. UAE has been widely implemented for oil extraction because of its ability to enhance the oil yield whilst preserving the quality of the oil (Thilakarathna et al., 2021). Furthermore, ultrasound base extraction enabled to replacement of toxic organic solvents that are mostly used in the oil extraction process with green solvents. Ultrasound is conveniently integrated with other extraction techniques, such as an enzyme, microwave, and supercritical technology to further enhance oil extraction. This review addressed to introduce the theoretical concept underlying ultrasound-assisted extraction, extraction mechanism as well as ultrasound extraction equipment.

Generally, ultrasound is defined as sound waves that have frequencies above those audible to the human ear, greater than approximately 20 MHz. However, lower sound wave frequencies that are typically used for the clinical purpose has frequencies between 2-12 MHz also considered as ultrasound. Depending on its frequency ultrasound can be used in different ways. In the medical field, ultrasound is used to create a picture of organs, tissues, or other organs in motion inside the human body, which is based on sound wave interaction with organic compounds. Extraction of bioactive compounds is one of the main processes in the food industry, conventional methods such

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as solvent, steam distillation, or mechanical pressing-assisted extraction are widely used in the food extraction process. All these extraction techniques are dependent on breaking low bounding energy between molecules, followed by formation of free space between molecules. Solvent-assisted extraction have several drawbacks, this technique required massive volume of toxic solvents which leads to waste generation, is technically and scientifically difficult to handle, complex and required long processing time, and needs a purification process to remove solvent and ensure safe final product consumption (Rodrigues & Fernandes, 2017).

Sound waves with a frequency between 20-400 kHz are categorized as ultrasound frequencies used in the food extraction process. Ultrasound waves induced by a vibration probe or bath transmitted vibration into the complex matrix (food) that can create voids and extract specific compounds, illustrated in Fig. 1 (Thilakarathna et al., 2021). In addition to ultrasound frequency, extraction efficiency is also determined by ultrasound amplitude, with higher amplitude forcing chemical bounding or cell wall to rupture (Senrayan & Venkatachalam, 2018).

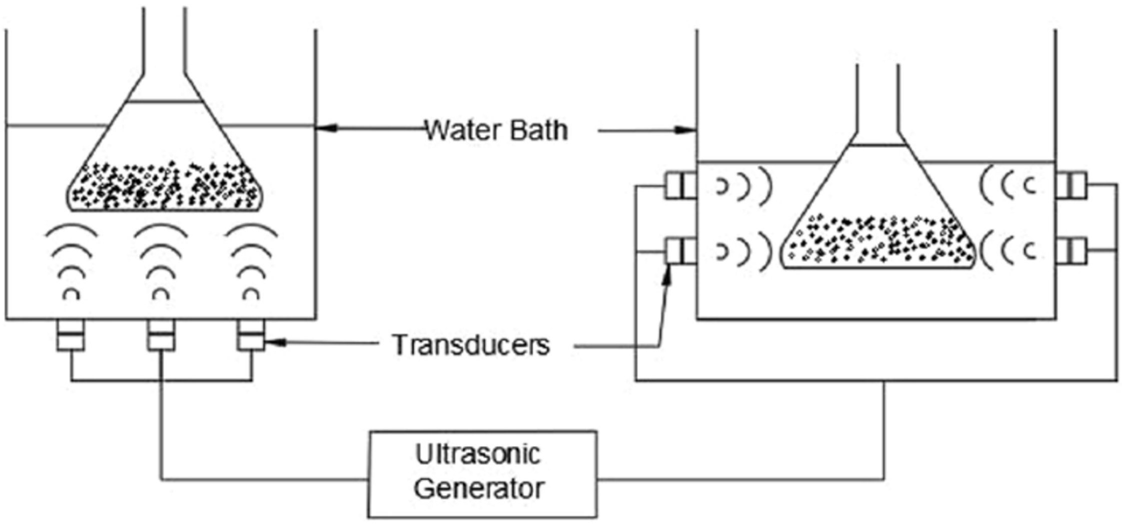

Figure 1. Schematic diagram of ultrasound-assisted extraction

As shown in Fig. 1, ultrasonic extraction offers several advantages compared to conventional methods, namely: required a simple instrument and easy setup, the absence of toxic solvent, low energy process, and less amount of solvent (Rodrigues and Fernande, 2017). Furthermore, several studies reported ultrasound extraction able to increase extraction yield (Rojo-Gutie´rrez et al., 2021; Senrayan & Venkatachalam, 2018; Stevanato & Silva, 2019).

Ultrasound waves

Extraction is a common process that is largely applied in food processing, extraction is conducted to separate specific compounds from a complex matrix such as seed or vegetable oil and fruit juices. The two most traditional extraction methods are forced extraction by applying pressure and solvent extraction which is based on the diffusion of the solvent into a solid matrix. These methods can be optimized by introducing a heating process that can increase yield extraction. Ultrasound-assisted extraction (UAE) has been intensively studied as a potential method to reduce processing time, temperature, and solvent consumption while able to increases yield extraction. Ultrasound waves are rapid longitudinal waves composed of alternating low and high pressure where their energy can compress and expand the compound of a solid matrix. This phenomenon is known as the sponge effect, a solid matrix squeezed when high pressure passes through it and rapidly released. This phenomenon happened repeatedly and depends on the bounding energy of the matrix compound, microscopic channels in the tissue will be created. This microchannel facilitates solvent diffusion into the solid matrix and forms a preferential pathway that provides the exit of the solubilized compounds from the solid matrix to the solvent phase (Rodrigues & Fernandes, 2017). Several studies have well reported the formation of the microchannel in fruit, Fig. 2 illustrates microchannels formed by ultrasound applied to strawberry tissue (Garcia-Noguera et al., 2010).

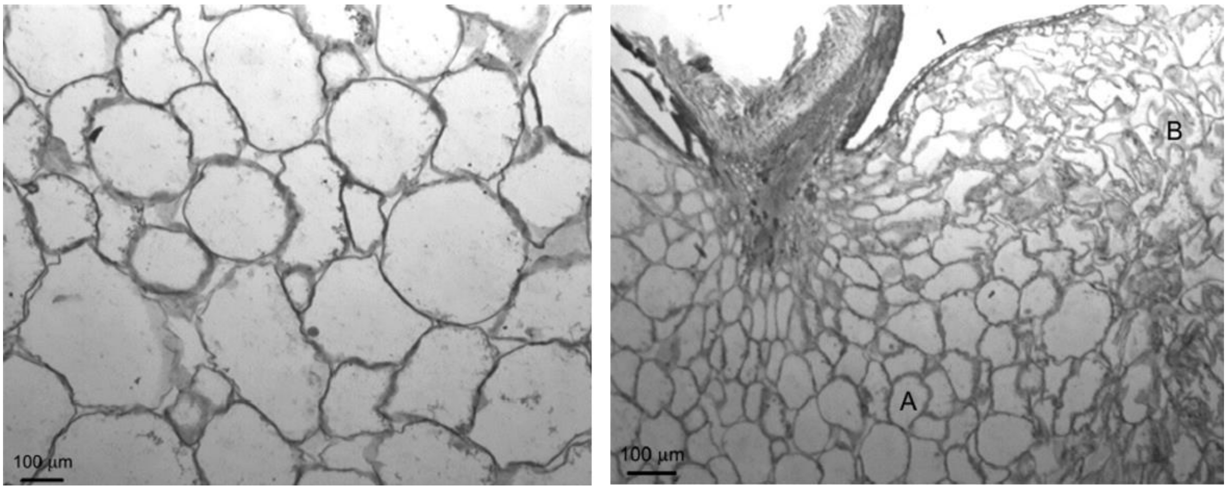

Figure 2. Effect of ultrasound application on strawberry tissue. (a). Cell cross sections from a thin layer of fresh strawberry tissue before ultrasound exposure, (b) Strawberry samples after 20 min of ultrasound exposure: (A) region with normal cells and (B) microchannel formation

Ultrasound extraction parameters, specifically frequency and amplitude, determined the maximum change of pressure of ultrasound wave that can generate microchannels and break down cell walls releasing the product into the solvent. Maximum pressure changes of sound wave expressed as follows:

$$\Delta P_m = (v\rho\omega)s_m \qquad (1)$$

Where v is the sound speed in the sample and ρ is the density of the sample, these two parameters are the intrinsic parameter of the target material. While ω is the angular frequency of a sound wave ($\omega = 2\pi f$) and s_m is the maximum amplitude of the sound wave, which are controllable parameters. The frequency and maximum amplitude of sound waves determine sonication energy that could provide the means to disrupt the cell wall of the solid material (Rodrigues & Fernandes, 2017). Heating and ultrasound exposure time is also reported able to enhance extraction efficiency, and in most cases, the extraction process of UAE is independent of the solvent properties (Garcia-Noguera et al., 2010; Rodrigues & Fernandes, 2017). Ranjan et al. (2010) reported that the application of ultrasound can increase the number of disrupted cells, thus providing high-yield product extraction. Furthermore, ultrasound-assisted reduces extraction time, temperature and solvent, and energy consumption (Paniwnyk et al., 2009; Pradal et al., 2016; Al-Dhabi et al., 2017).

Ultrasounds have a positive and negative effect on target bioactive compounds depending on the intrinsic and extrinsic parameters of ultrasound treatment. Mainly ultrasound-assisted extraction efficiency depends on treatment time, ultrasound energy (frequency and maximum amplitude), and the solvent and solid ratio. Careful design of ultrasound extraction plays a pivotal role to determine extraction quality, over-processing may lead to the degradation of bioactive compounds. As an example, less stable compounds such as polymers, vitamins, and enzymes may decompose at high energy and long exposure ultrasound which may lead to a decreasing extraction yield. While the extraction yield of the stable compound, such as phenolic continuously increases when subjected to ultrasound waves, illustrated in Fig. 3 (Rodrigues & Fernandes, 2017).

Ultrasound-assisted Extraction

The UAE has been applied to natural products using different chemicals and parts of the plant. Some research suggested that the use of ultrasound during extraction would increase the yields, especially for the bioactive compounds. The UAE would be beneficial to extract pectin, polysaccharides, dietary fiber, polyphenols, and fat (oil). Optimization of the UAE method can be performed by adjusting some variables, for example, wave frequency, time, pH, as well as the ratio of solute and solvent. Besides, these waves consist of a series of compression and rarefaction that can propagate through solid, liquid, or gaseous media in reducing the displacement or release of molecules from their initial position. The negative pressure during the rarefaction of high-intensity sound waves exceeds the attractive forces that link the molecules together and pull them apart creating cavitation bubbles. These bubbles grow through coalescence and then collapse during the compression phase creating hot spots and extreme local conditions. The temperature can reach up to 5000 K and the pressure increase can reach 1000 atm. These hot spots speed up the surrounding biochemical reactions (Kumar et al., 2021).

Quiroz et al. (2019) also observed a significant difference in the optimization results with the independent variables seed-solvent ratio and extraction time. This is influenced by the ionization state of the aliphatic molecule on pH modification and the physical process of mass transfer associated with the effect of ultrasound as well as solvent concentration. Bixin is a carotenoid that has a highly conjugated structure and presents a carboxyl group. This causes bixin to be very soluble at alkaline pH, whereas polyphenolic compounds are slightly acidic molecules, and their solubility is not greatly affected by the pH of the medium. Therefore, the solvent concentration (ethanol) is the most influential variable. The results of the statistical analysis of the seed-solvent ratio were influenced by the saturation of the extraction medium. Furthermore, the importance of the treatment time during the extraction process is related to the residence time of the cavitation bubbles in contact with the material assisting in the release of metabolites. In ultrasound treatment in the first 15 minutes, the mass transfer is mainly influenced by convection and according to the extracted solute, the diffusion phenomenon that occurs involves the internal part of the seed particles where compound extraction is maximized. Moreover, extraction using UAE method reduces the extraction time and solvent volume compared to extraction using soxhlet. The UAE also increases the levels of quercitirin and total phenol of *C. caudatus* leaves (Latiff et al., 2021).

Cavitation on ultrasound-assisted extraction

Ultrasound cavitation or acoustic cavitation has long been demonstrated in aqueous systems. This stems from the thought of negative pressure in the sound waves breaking the liquid, but this is highly unlikely theoretically. It is highly improbable because it would produce a minimum pressure and require at least hundreds of atmospheres which is not available in sound waves. The approach that can be given partly comes from the fact that small impurities in the liquid, such as suspended particles have a negligible effect compared to the very small concentration of microbubbles. Microbubbles differ from solid particles, simply increasing the compressibility of liquids, introducing a form of loss of viscosity and non-reversible energy exchange that is not present in solid particles (Arrigo, 2011).

Cavitation can be defined as tiny gas bubbles that grow, oscillate, and collapse in a liquid when affected by ultrasound waves. This cavitation is influenced by the physical properties of the solvent such as viscosity, saturation vapor pressure, and surface tension. Cavitation formation is required to apply negative pressure during the refining cycle (rarefaction) large enough to disrupt the natural cohesive forces of the solvent to form a vacuum. The increase in viscosity also intensifies the molecular interactions thereby inhibiting cavitation. The phenomenon of cavitation is also affected by high surface tension and vapor pressure (both temperature related). It takes 105 kPa to initiate cavitation in pure water. Temperature can also affect cavitation by changing the vapor pressure, viscosity, and surface tension of the solvent. As the temperature approaches the boiling point of the solvent, cavitation bubbles are generated more easily than at low temperatures, but the explosive capacity is reduced. High temperatures also increase the rate of solvent diffusion and mass transfer. Therefore, the temperature of the solvent should be controlled during the UAE. Frequency and intensity play an important role in cavitation. The frequency commonly used for extraction is 20-40 kHz. A short delay is required during the refining phase for cavitation bubbles to form. High frequencies adversely affect cavitation, because the higher the frequency, the shorter the refining phase, making the formation of cavitation bubbles difficult. Therefore, high-frequency ultrasound is said to be non-destructive because the frequency is too high to allow for the phenomenon of acoustic cavitation. Moreover, cavitation bubbles can only be generated when the pressure applied to the medium (PL) drops below the saturated vapor pressure of the liquid (PV). The pressure applied to the medium under ultrasound results from the sum of the hydrostatic pressure and the acoustic pressure. Therefore, the higher the pressure applied to the medium, the more difficult it is to induce cavitation. The ultrasonic pressure and intensity must be increased to allow cavitation (Maryline & Chemat, 2020). The formation of cavitation bubbles during UAE was illustrated in Figure 4.

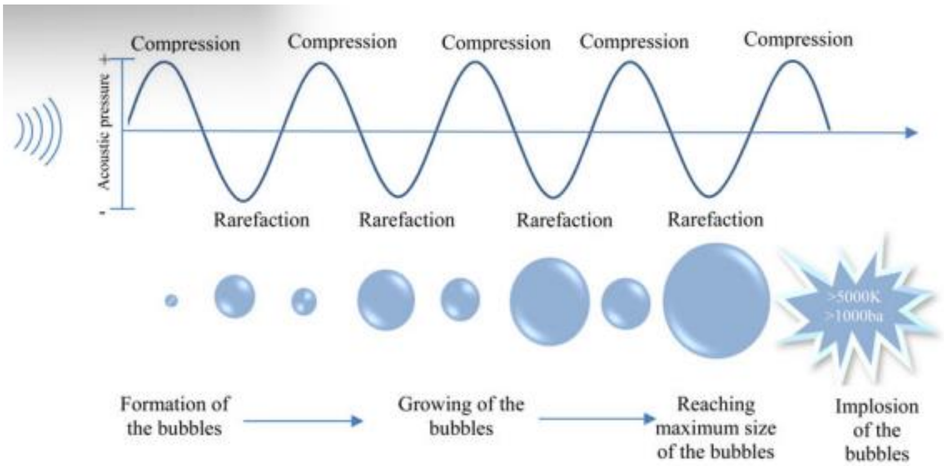

Figure 4. The formation of cavitation bubbles in UAE (Bui et al. 2020)

Advantages and disadvantages UAE system

Two UAE operating systems provide direct and indirect effects. The direct system usually uses an ultrasonic probe, while the indirect system uses an ultrasonic bath. The ultrasonic bath system has an ultrasonic sounder that provides an excitation signal according to the modal frequency of the tuning assembly. Electrical energy is converted into mechanical vibration by a piezoelectric transducer. The tuning system sends ultrasonic waves generated by mechanical vibrations to the air medium in non-contact applications. Ultrasound transmission is largely dependent on the acoustic characteristic impedance of the propagation medium. Therefore, the difference between the impedance of the ultrasonic transducer material and air has a great influence on the application of non-contact ultrasonic waves (Wen et al., 2018). According to Carreira-Casais et al. (2021), bath systems are more economical for ultrasound irradiation. The equipment needed is a transducer which is under a stainless tank with a continuous system. The advantages of this system are lower costs and uniform energy distribution in the vessel which does not require adaptation of the reaction vessel. The disadvantage of this system is that the recovery of bioactive components bound to complex matrices such as algae (algae) is still a challenge since the strength must be sufficient to produce cavitation in the extraction vessel. An important aspect that needs to be considered is the lack of adequate temperature and power control which causes a lack of efficiency in the vessel containing the extract.

The extraction efficiency of bioactive compounds can be achieved using an ultrasonic probe system, or what is commonly called a direct contact system. Another research conducted by Wen et al. (2018) indicated that This system has been widely used for the sonication of small samples. The probe-type ultrasonic is more powerful than the ultrasonic bath. Probe-type ultrasonics improve mass transfer, but it is also necessary to pay attention to the degree of sample destruction caused by the degradation of the ultrasound probe. The uniformity of the applied pressure field in the equipment needs to be considered to calculate the optimal reactor size and element position relative to the sensor, to obtain maximum energy transfer to the fluid. It should also be noted that the temperature of the sample will increase sharply during sonication, so the design of the sonication device needs to be considered. In addition, the main advantage of ultrasonic probe systems is that the extraction process can be optimized in terms of several parameters such as amplitude, time, and pressure to ensure that the molecular structure is not damaged. In this case, the operating temperature (T) can be reduced which allows for temperature-sensitive components (Carreira-Casai et al. 2021). Figure 5 showed the differences ultrasonic bath and the probe.

Mechanism of extraction of bioactive compound and parameters

Acoustic cavitation is the main mechanism in the UAE. Collapsed cavitation bubbles and sound waves can induce any one or a combination of the phenomena of fragmentation, local erosion, pore formation, shear forces, increased absorption, and swelling index in the plant cellular matrix. Collapsed cavitation bubbles generate shock waves and accelerated collisions between particles causing fragmentation in the cellular structure. The rapid fragmentation causes solubilization of the bioactive components in the solvent due to a decrease in particle size, an increase in surface area, and a high mass transfer rate at the boundary of the matrix layer on the surface (Kumar et al., 2021). Several things must be considered when using the UAE in. This is done to achieve the objective of the extraction process, where yield is not the only goal of the extraction process, but also the use of non-renewable resources in line with low energy consumption (Chemat et al., 2017). Some of the parameters that need to be considered to achieve these goals will be described as follows.

Duration of extraction

During the UAE process, the solvent will interact with the sample or solute. The extraction efficiency is affected by the interaction time of the two phases. There are two phases, namely the "washing" phase and the "slow extraction" phase. The first step, namely the “washing” stage is operated in the first 10-20 minutes of extraction during which the surface matrix containing the solute of the solute component will be carried out, and about 90% of the extraction can be carried out at this stage, resulting in a fast extraction rate. The second phase is called "slow extraction", where the diffusion process occurs in 60-100 minutes as mass transfer of the solute diffuses into the solvent. An increase in the sonication time increases the initial yield and then the yield decreases with a further increase in time. This effect is similar to an increase in power and temperature. The initial increase in sonication time causes the cavitation effect of ultrasound to increase swelling, hydration, fragmentation, and pore formation of plant matrix tissue from which solutes are extracted. All these factors increase the exposure of the solute and the extraction medium to aid its release into the solvent. Prolonged exposure to ultrasound causes structural damage to the solute and reduces extraction yield (Kumar et al., 2021).

Temperature of extraction

Temperature greatly affects the solvent. An increase in temperature can decrease the viscosity and surface tension and induce an increase in vapor pressure. The increase in vapor pressure causes the more solvent vapor to enter the bubble cavity and many cavitation bubbles will collapse lighter (less violently) thereby reducing the sonication effect (Chemat et al., 2017). Also, Carreira-Casais (2021) suggested that temperature is a two-sided parameter that plays an important role in the UAE. Temperature is closely related to the nature of the solvent because it directly affects the properties of the solvent. The increase in temperature includes a reduction in solvent viscosity and surface tension, but an increase in the vapor pressure of the solvent is also accompanied by an increase in the quantity of gas entering the bubble and reducing the collapse expansion. Therefore, the high temperature does not increase the yield of extractive compounds from the matrix in the ultrasonic device. An increase in temperature can also favor the extraction of compounds with a diffusion rate of the binder and disrupt the external chemical bonds of the matrix, thereby aiding release to the medium, but an increase in temperature can also hurt the quality of the extracted material. Therefore, optimization of the extraction temperature should be carried out to improve the extractive properties of the solvent used in the UAE and protect the structure and function of the target components.

Types of solvent

Each solvent has its specific gravity and polarity. The nature and polarity can affect the amount of extract yield. It is important to use a certain solvent that could affect the significant effect of cavitation during acoustic energy transfer to the reactant. Kumar et al. (2021) also stated that some solvents that have been used for extraction with the UAE are acidified water, ethanol, alcohol, acetone, water, and so on. The solvent used for UAE pectin is acidified water, water acidification is carried out using mineral acid or the use of citric acid. Mineral acids that have been used to convert distilled water were evaluated for their effect on pectin extraction including hydrochloric acid (grapefruit peel, durian peel), and nitric acid (for passion fruit peel, manganese peel). Citric acid has been used for grapes, pomace, banana peels, orange peels, and sunflower heads for UAE from pectin. Alcohol and ethanol with different water content have been widely used for phenolic compounds from plant materials. Ethanol was found to have the highest affinity for phenolics in many systems, in addition, ethanol was used as the solvent of choice due to its affordability, derived from renewable sources (sugarcane) and its categorization as a solvent which is GRAS (Generally Recognize as Safe).

Ultrasonic power of extraction

The power delivered during the UAE has been expressed as a percentage amplitude in the range of 0-100% where the 100% amplitude represents the rated power of the equipment and the power density (W.m/L) which is calculated as the power dissipated per unit volume of the extraction medium (solvent). There is a linear relationship between ultrasonic amplitude and output power when the amplitude is between 30% to 80% of the maximum power. The power applied by the UAE to the bioactive by-products of UAE-treated fruits and vegetables depends on the compound to be extracted and the type of plant matrix selected with variations in the 20-40 W range (Kumar et al., 2021). Several studies have shown that high ultrasonic power causes material changes by inducing larger shear forces (depending on the nature of the material and solvent medium), but in the food industry, this parameter is usually optimized to use minimum power to produce the best results (Chemat et al., 2017).

Conclusion

Considering all the above-mentioned factors, ultrasound base extraction enabled to replacement of toxic organic solvents that are mostly used in the oil extraction process with green solvents. The UAE has been applied to natural products using different chemicals and parts of the plant. Some research suggested that the use of ultrasound during extraction would increase the yields, especially for the bioactive compounds. The UAE would be beneficial to extract pectin, polysaccharides, dietary fiber, polyphenols, and fat (oil). Optimization of the UAE method can be performed by adjusting some variables, for example, wave frequency, time, pH, as well as the ratio of solute and solvent. As the main mechanism in the UAE, collapsed cavitation bubbles generate shock waves and accelerated collisions between particles causing fragmentation in the cellular structure.

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