

ULTRASOUND ASSISTED HYBRID TECHNOLOGIES FOR EXTRACTION OF BIOACTIVE COMPOUNDS

ABSTRACT

The sustainable and efficient extraction of bioactive compounds from natural resources offers a promising alternative to synthetic additives and formulations. Conventional extraction methods often exhibit drawbacks such as high energy consumption, excessive solvent usage, prolonged processing times, low extraction yields, and negative environmental impacts. To address these limitations, innovative extraction technologies have emerged, providing more sustainable and efficient solutions. Ultrasound is a promising technology used for extraction of these beneficial compounds with enhanced yield and quality. It can assist various other extraction technologies including microwave assisted extraction, ohmic heating, enzyme extraction, subcritical water extraction and supercritical fluid extraction to obtain high quality extracts. This synergistic approach results in increased extraction yields and improved product quality by reducing extraction time, minimizing solvent consumption, and utilizing moderate energy inputs.

Keywords: ultrasound, hybrid technology, extraction, bioactive compounds

1. INTRODUCTION

Bioactive compounds are phytochemicals present naturally in food which can modulate metabolic processes and provides better health benefits beyond the basic nutritional value of food (Galanakis, 2017). The antioxidant, anticarcinogenic, anti-inflammatory, and antimicrobial characteristics of these compounds render them highly demandable by the food, pharmaceutical, nutraceutical, cosmetic, and textile industries. Extraction of these beneficial compounds using different technologies develops various valorised products, including functional foods or dietary supplements. The selection of extraction method is influenced by the required purity of extract, physical and chemical properties of the target compound, its location, cost-effectiveness and the overall value of the extracted product (Patra *et al.*, 2022).

Conventional methods for extracting bioactive compounds, such as maceration, percolation, pressing, solvent extraction, hydro-distillation, steam distillation, and steam diffusion, exhibit high energy consumption, excessive solvent usage, prolonged extraction times, low extraction yields, and negative environmental impacts. To circumvent these issues, a multidisciplinary approach named "Green extraction" was aimed to develop sustainable extraction technologies which saves time and energy, increases extraction yield, reduces solvent consumption, yields quality extract, and valorise natural resources (Chemat *et al.*, 2012; Chutia and Mahanta, 2021). Additionally, the use of green solvents creates a way to have the lowest possible impact on environment. The selection of green technologies is based on their benefits, drawbacks, operating principles, and available extraction equipment. The major green extraction technologies are ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), pulsed electric field assisted extraction (PEF), ohmic heating assisted extraction (OH), subcritical water extraction (SWE), and supercritical fluid extraction (SFE) (Chemat *et al.*, 2012; Capaldi *et al.*, 2024). Ultrasound has got a special interest due to its sustainable impacts on bioactive extraction such as higher yield, low solvent, time, and energy consumption, and is a promising technology. These sound waves extract bioactive compounds using high intensity waves, which causes disruption of cell walls by a phenomenon called cavitation that enhances the mass transfer in a very less time. Combining ultrasonic technology with other novel processing methods holds

great promise for improving the extraction of bioactive compounds from variety of food sources (Wen *et al.*, 2018; Yusoff *et al.*, 2022).

2. ULTRASOUND

Ultrasound are mechanical waves at a frequency above the threshold of human hearing (>16 kHz) (Chemat *et al.*, 2011). Its application varies according to the frequency ranges. The sound waves with frequencies greater than 100 kHz and low power ($< 1\text{Wcm}^{-2}$) constitute low intensity ultrasound, which finds applications in analytical techniques such as non-destructive analysis. The high power, low frequency (16-100 kHz) sound waves are high intensity ultrasound which could alter the properties of food either physical or chemical. The high intensity ultrasound offers a simple and efficient approach to

enhancing the extraction of bioactive compounds (Ashokkumar, 2015; Rutkowska *et al.*, 2017; Soria and Villamiel, 2010).

2.1 Mechanism

The working principle is based on the phenomenon named as cavitation. When the sound waves propagate through a medium, they induce a series of compression and rarefaction waves on the molecules (Soria and Villamiel, 2010). During the rarefaction phase, a negative pressure will act on the molecules, causing them to pull apart. When the power reaches a significant level, the rarefaction cycle might surpass the attractive forces among liquid molecules, leading to the formation of cavitation bubbles originating from pre-existing gas nuclei in fluid. The cavitation bubbles are mainly two types; stable and transient. The stable bubbles oscillate many times, growing and shrinking but stays roughly the same size. In contrast, transient bubbles exist only for few acoustic cycles. These bubbles, dispersed throughout the liquid, gradually enlarge over a few cycles until it reaches a critical size, at least double their initial size. At this point, they become unstable and implode violently during a compression cycle (Chemat *et al.*, 2017).

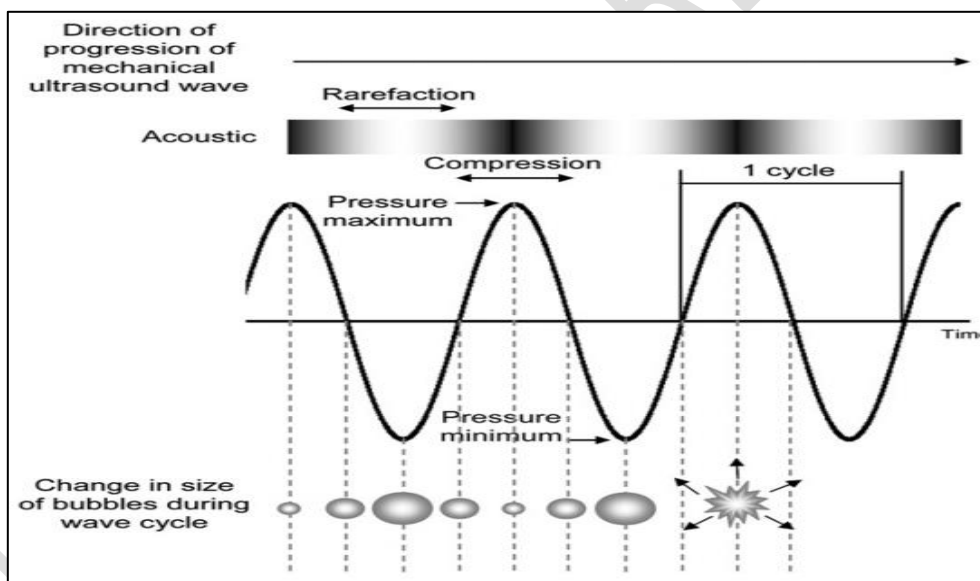


Fig. 1. Ultrasonic cavitation (source: Soria and Villamiel, 2010)

When cavitation bubbles implode, they create intense localized hotspots with extreme temperatures (5000 K) and pressures (1000 atm) as shown in Fig. 1. These intense conditions generate shockwaves and turbulent flow within the cavitation zone, potentially causing significant damage and other effects. Moreover, the changing size and collapse of cavitation bubbles trigger powerful micro-streaming currents. These currents involve rapid changes in velocity and intense shearing forces, ultimately altering the properties of the surrounding medium (Soria and Villamiel, 2010).

2.2 Ultrasound systems

The two major types of instruments for the extraction are ultrasound bath and probe as shown in Fig. 2. These systems improve the process of extraction with high reproducibility, reduced solvent consumption, simple operation, production of high-quality product, wastewater elimination and reduced fossil energy consumption when compared to conventional extraction processes. Ultrasound baths, typically consisting of a stainless steel tank connected to a transducer, are commonly employed for dispersing solids into solvents. They are less used for chemical reactions due to its low reproducibility,

but is economically advantageous and easy to handle. The probe system is much more powerful and is widely used for sonication of small volumes of sample. It is dipped in a vessel and delivers the waves with reduced energy loss. However, special care must be taken to mitigate the risk of sudden increase in temperature (Chemat *et al.*, 2017).

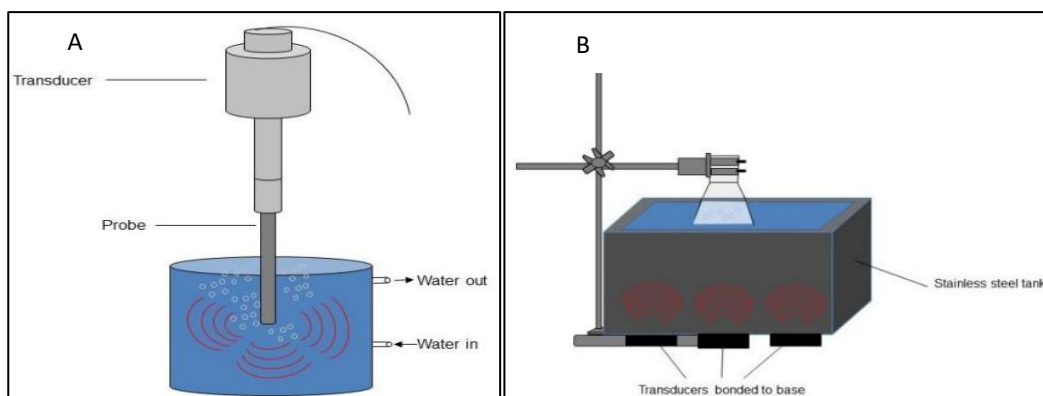


Fig. 2. Ultrasonic systems: (A) ultrasonic probe (B) ultrasonic bath

3. ULTRASOUND ASSISTED EXTRACTION

Ultrasound Assisted Extraction (UAE) has been demonstrated to be an effective and eco-friendly technology. It works on the principle of acoustic cavitation that alter the properties of cell structure by disruption, thus enhancing the bioavailability of phytochemicals (Singla and Sit, 2021). All the mechanical and physical effects (micro-jets and shock waves) generated by cavitation accelerates the fragmentation, sono-poration, shear force and turbulence, and localised damage to plant tissues, causing an increased mass transfer allowing maximum penetration of solvent into the sample matrix (Soria and Villamiel, 2010; Kumar *et al.*, 2021). Increased mass transfer caused by the extreme temperature and pressure conditions results in cell disruption and thinning of membrane layers (Khadhraoui *et al.*, 2021). It improves the extraction efficiency of the targeted compound by increasing the extraction yield and reducing the time (Wang and Weller, 2006; Nayak and Rastogi, 2013).

3.1 Effect of process parameters

The major parameters influencing the extraction are power, frequency, time, solvent to solid ratio, temperature, and solvent. These needs to be in precise control for the optimum extraction to be carried out (Kumar *et al.*, 2021).

3.1.1 Ultrasonic power

The extraction yield increases with increasing power until a peak value is reached, beyond which it decreases due to violent cavitation. The bubble size is proportional to power and higher power weakens the effect of cavitation by producing larger cavitation bubbles which hinder the mass transfer and reduces the yield. The effect of power also depends on parameters such as temperature, and time (Liao *et al.*, 2016; Kumar *et al.*, 2021). This was observed in a study conducted by Arruda *et al.* (2019) that there is a significant increase in the phenolics and antioxidant activity with increasing power and time. However, prolonged exposure to high ultrasonic powers can lead to the degradation and structural destruction of phenolics due to higher temperature and pressure generated by the vigorous cavitation, leading to lower extraction efficiency. To mitigate this, maintaining lower extraction time and temperature ($\leq 40^{\circ}\text{C}$) through cooling the product, helps to prevent overheating and the degradation of extracted compounds.

In extraction of polyphenols from Chinese propolis, an increasing trend in phenolic compound was observed with increasing power, up to 135 W. At a particular range of energy, the increase in power and cavitation amplifies the destruction of cell matrices leading to enhanced extraction. Highest phenolic content was observed at 135 W, beyond which the yield decreased, as depicted in Fig. 3. This may due to the degradation of polyphenols with higher energy input (Peng *et al.*, 2023).

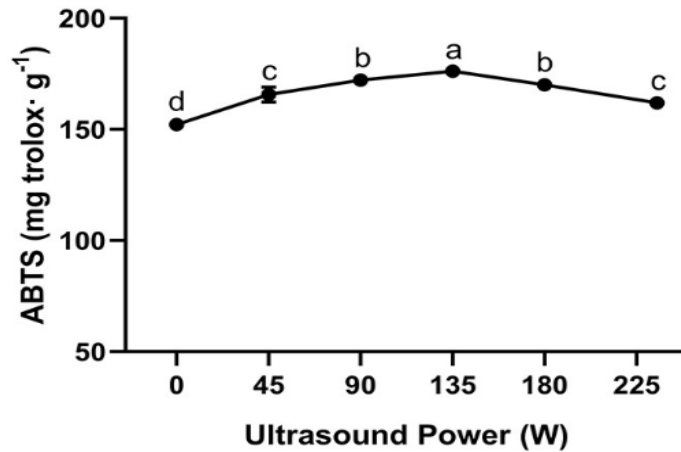


Fig. 3. Effect of power on the antioxidant activity (ABTS assay) of propolis extract (source: Peng *et al.*, 2023)

3.1.2 Frequency

Mostly the ultrasound extraction systems are working at a particular frequency, but some are providing multiple frequencies. Frequency increases with increase in intensity to achieve the desired cavitation. However, cavitation intensity tends to decrease with increasing frequency (Zia *et al.*, 2022). Low frequency creates a smaller number of cavitation bubbles with larger diameter providing large cavitation effect which reduces with increase in frequency. At higher frequency the bubble gets shorter time for growing which obstructs their implosion effect. The resistance to mass transfer increases with increase in frequency producing large number of bubbles (Kumar *et al.*, 2021).

In the extraction of quercetin and rutin, the yield was observed to increase with frequency from 20 to 80 kHz. Further increases in frequency resulted in a gradual decline in yield, as illustrated in Fig. 4 (Liao *et al.*, 2016).

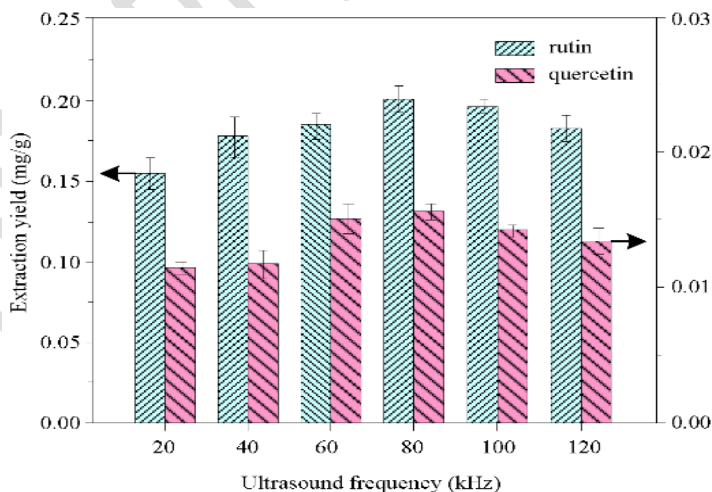


Fig. 4. Effect of ultrasound frequency on the extraction of quercetin and rutin

(source: Liao *et al.*, 2016)

3.1.3 Ultrasonic time

The increase in sonication time enhances the extraction yield initially and then decreases the yield with further increasing the time. The initial increment in time resulted in enhanced cavitation effect causing an increased release of compounds. Long time exposure of ultrasound causes structural damage to solute thus reduces the yield (Kumar *et al.*, 2021).

Cheung and Wu (2013) observed that yield of polysaccharides increased rapidly with time from 10 to 20 min, and followed by a decreasing trend. Similarly Dash *et al.* (2021) reported a rapid increase

in oil yield from in *T. chebula* kernels from 5 to 30 min and further increment slow down the extraction and got stabilised. It is described that ultrasonication causes the fragmentation and disintegration of plant matrix which increases the contact area between solvent and solid interface. After the initial increase the extraction rate decreases due to the less movement of extracts through diffusion and osmotic mechanism. In the extraction of antioxidants from kiwifruit, the long-term extraction led to the degradation of antioxidants as shown in Fig. 5. Thus, the optimum time extraction was observed as 30 min (Mai *et al.*, 2022).

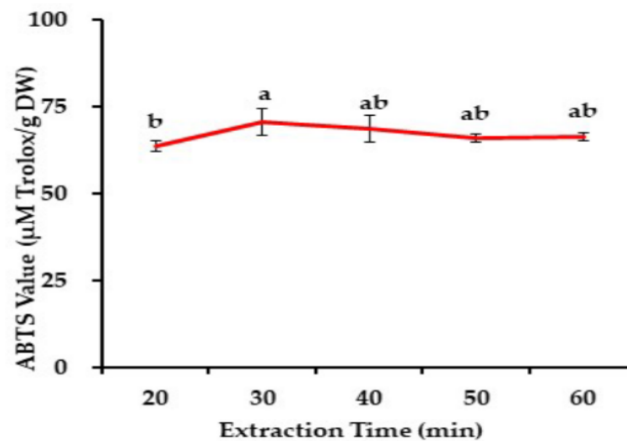


Fig. 5. Effect of extraction time on ABTS assay reducing capacities of kiwifruit

(source: Mai *et al.*, 2022)

3.1.4 Solvent to solid ratio

A proper solvent to solid ratio is necessary for enhancing the extraction rate (Peng *et al.*, 2023). The yield increases with increase in the solvent to solid ratio up to a certain level and then decreases after reaching a peak value. At lower ratios, the high viscosity of the solution hinders cavitation, as a significant negative pressure is required to overcome viscous forces. With the initial increase in ratio, the cavitation effect is greater due to the decrease in viscosity and concentration, resulting in an increased yield. At very high ratios, the yield is reduced by the degradation of desirable solutes resulting from excessive cavitation (Kumar *et al.*, 2021).

Liao *et al.* (2016) investigated the effect of solvent to solid ratio on the extraction of quercetin and rutin. With an increase in ratio from 20 to 40 mL/g, a rapid increase in the yield was obtained. It was inferred that, more solvent volume effectively dissolves the target compounds. Despite that, further increase in solvent volume showed no significant increase in yield. Dash *et al.* (2021) reported maximum oil yield of 29.16%, 32.7%, and 36.75% obtained for solvent to solid ratio of 5:1, 17.5:1, and 30:1, respectively. Higher ratio increases the solvent volume, causing uniform mixing and flow into the core of particle. This creates an enhanced concentration gradient between solid and solvent leading to a greater release of oil. Peng *et al.* (2023) studied the extraction of polyphenols from Chinese propolis, observing an increasing trend in total phenolic and flavonoid content with increasing solvent-to-solid ratios from 20:1 to 60:1 (v/w). Further increases in the ratio either decreased the level or remain the same as shown in Fig. 6.

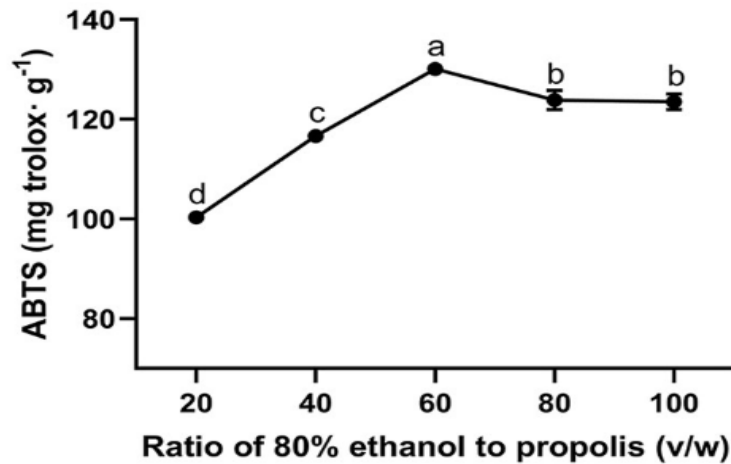


Fig. 6. Effect of solvent-solid ratio on the antioxidant activity (ABTS assay) of propolis extract (source: Peng *et al.*, 2023)

3.1.5 Temperature

The yield increases with increase in temperature up to a point and then it decreases. Increasing temperature enhances the solubility of solutes in the solvent and reduces solvent viscosity, leading to an increased diffusivity of solvent in the matrix. Further increase weakens the impact of cavitation and decreases the yield (Kumar *et al.*, 2021). Thus, low temperature is favourable for effective cavitation and temperature control is essential (Chemat *et al.*, 2017). This trend was shown in the study conducted by Liao *et al.* (2016) in which the extraction yield of quercetin and rutin increased with an increase in temperature from 20 to 30°C. After 30°C, the yield reduced due to the gradual decomposition of compounds as shown in Figure 7.

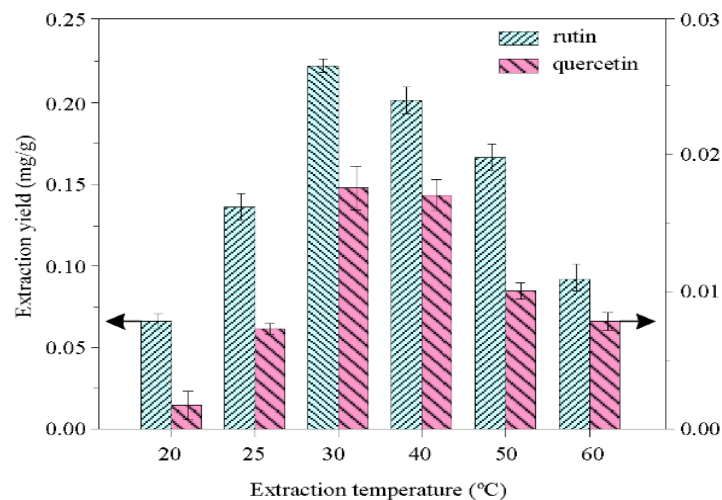


Figure 7. Effect of extraction temperature on the extraction of quercetin and rutin (source: Liao *et al.*, 2016)

3.1.6 Solvents

Solvent choice is based on the target metabolites and the physical parameters including viscosity, surface tension and vapour pressure. Solvents with high viscosity or surface tension increase the resistance to cavitation. Conversely, low vapour pressure solvents are more preferred, as they exhibit more intense cavitation compared to those with higher vapor pressure. The vapour pressure is also influenced by the temperature of the medium (Chemat *et al.*, 2017). Common solvents are ethanol, ethyl acetate, methanol, and acetone. Ethanol is the most commonly used solvent in UAE due to its

renewable nature and GRAS (Generally Recognised as Safe) status. It has high affinity towards phenolic compounds (Kumar *et al.*, 2021).

4. ULTRASOUND ASSISTED HYBRID TECHNOLOGIES FOR EXTRACTION

Integration of two technologies could enhance the overall extraction process by addressing the limitations of each individual approach. Employing multiple technologies simultaneously enables their advantages to be merged into a single process, thereby minimising the space, time and energy required in contrast to a sequential connection. In addition, the combination of technologies require high initial investment and energy demands, but is highly efficient and provides substantial benefits to offset these initial costs (Capaldi *et al.*, 2024).

4.1 Ultrasound assisted microwave extraction

Microwaves are non-ionizing electromagnetic waves having frequency in the range between 300 MHz-300 GHz. The principle of microwave heating is based on ionic conduction and dipole rotation. The vigorous vibrations and collision of molecules caused by these phenomena develops frictional force which in turn generates internal volumetric heating. The heat energy generated evaporates the moisture leading to a pressure generation which ruptures the cell wall. This facilitates the release of active compounds from the cells to the surrounding solvent by cell wall expansion and disruption (Mandal *et al.*, 2007; Bagade and Patil, 2021). The biomaterial will be subjected to internally generated heat only for a short duration leading to increased quality of extracted oil.

Microwave assisted extraction (MAE) enhances extractability by modifying the cell structure offering advantages such as increased extraction rates, higher yields, reduced time, and lower solvent consumption over conventional methods (Pongmalai *et al.*, 2015; Nithya *et al.*, 2023). However, this technique may lead to thermal degradation of sensitive bioactive compounds. Ultrasound pretreatment could be applied to modify the cell structure without any damage to thermally unstable compounds (Wang and Weller, 2006). The combined treatment could be considered as a non-thermal treatment with moderate energy consumption and low cost. The cavitation impact of ultrasound breaks down the plant matrix facilitating solvent penetration, while the fast and efficient heat exchange of microwave enhances the mass transfer, leading to an improved yield with high quality. The hybrid ultrasonic microwave bath reactor is shown in Fig. 8.

Pongmalai *et al.* (2015) investigated the effect of cell structure modification in cabbage for the extraction of bioactive compounds from the outer leaves by the application of ultrasound as a pretreatment prior to MAE. The microstructural analysis shows that, by the combination of ultrasound and microwave, the cell structure suffered extensive damage due to the combined effect of cavitation and rapid heating. The study concluded that this synergistic approach enhanced the extractability due to the extensively damaged structure.

Zhang *et al.* (2023) also demonstrated the effect of Ultrasound Assisted Microwave Extraction (UAME) on the yield, chemical structures, and antioxidant activity of *Dictyophora indusiata* polysaccharides (DP). The study revealed that UAME treated sample exhibited a greater degree of surface collapse and folds compared to other methods, facilitating faster cell dissolution. Therefore, the application of ultrasound causes the rapid, uniform, low temperature extraction. The polysaccharides extracted using UAME showed the highest antioxidant value, it is due to the higher exposure of more compounds and the decrease in molecular weight. Thus, this complementary technology not only reduced the extraction time, but also produced extracts with higher antioxidant value and overcomes the shortcomings of both technologies.

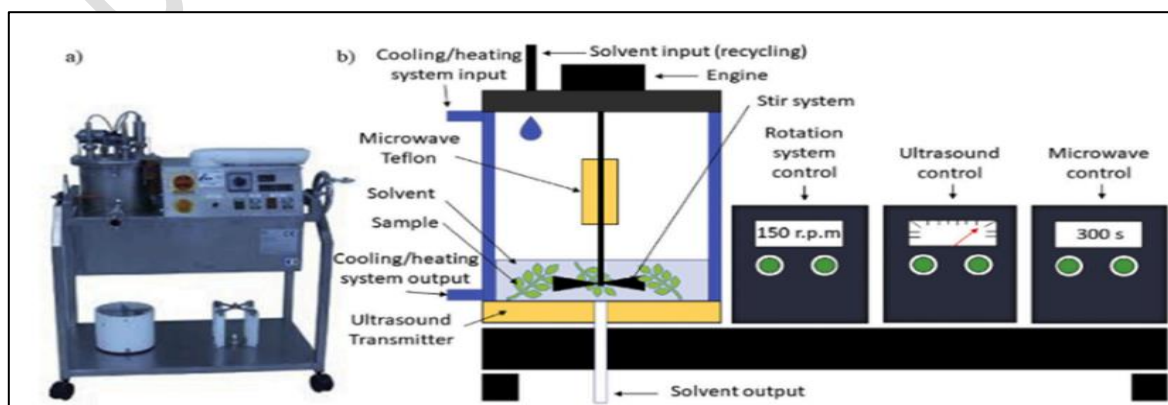


Fig. 8. Hybrid system of microwave and ultrasound bath reactor (Source: Ojha *et al.*, 2020)

4.2 Ultrasound assisted enzyme extraction

Enzyme assisted extraction of bioactive compounds is a promising alternative to conventional extraction methods offering an efficient, sustainable, and eco-friendly approach. It depends on the characteristic property of enzymes to carry forward reactions with accurate specificity, region-selectivity, and the ability to operate under mild conditions while retaining the biological potencies of bioactive compounds. Commonly used enzymes include cellulase, pectinase, hemicellulases, and proteases.

The basic principle is hydrolysing the plant cell walls using specific enzyme catalyst under optimum experimental conditions, which disintegrates the cell wall and improves the permeability to facilitate the release of components. The plant cell wall binds to enzyme which causes the maximum interaction between cell wall and enzyme. Enzymes change their shape, causing the bond-breaking of the cell wall, thereby releasing the active constituents from it. It provides better yield under controlled temperature and simple recovery with reduced solvent and energy consumption. Particle size, time, pH, and temperature are major parameters to be considered. Efficient extraction at moderately low temperature and pH within a short period eliminates the requirement of expensive equipment (Lubek-Nguyen *et al.*, 2022). The major drawbacks are high cost of enzyme and the inability of enzymes to completely hydrolyse the bonds in cell wall, thereby limiting the release of active constituents and reducing yield (Liu *et al.*, 2016; Nadar *et al.*, 2018).

The synergistic effect of ultrasound and enzymes accelerate the release of intracellular substances leading to increased yield and reduced extraction time. Ultrasound, through its mechanical effects and cavitation, disrupts cell walls, increasing surface area and facilitating mass transfer, making the cell contents more accessible to enzymes. When the enzyme gets subjected to optimum ultrasound condition, it undergoes favourable conformational changes leading to an increased activity. This helps in reduced use of enzyme for extraction, thereby reducing the total cost (Capaldi *et al.*, 2024).

Chen *et al.* (2014) demonstrated that ultrasound can reduce the molecular weight of polysaccharides by cleaving chemical bonds, enhancing their physicochemical properties. Also, the enzyme hydrolyses the cell wall to promote the dissolution of polysaccharides. Lin *et al.* (2023) extracted polysaccharides using Ultrasound Assisted Enzyme Extraction (UAEE) and resulted in a higher yield accompanied by an elevated antioxidant activity. Yun *et al.* (2023), also utilized UAEE to extract *S. baicalensis* root polysaccharide in which ultrasound improved the enzymatic activity and cellulase found to have significant impact on the yield under ultrasonic environment. The study also concluded that, the optimization of ultrasound treatment is essential, or else the use of high ultrasound power will reduce the yield by causing structural damage to polysaccharides.

4.3 Ultrasound assisted ohmic extraction

Ohmic heating (OH) is an electrical resistance heating by the flow of an alternating electric current through the foods (Goullieux and Pain, 2014). The electrical resistance of food causes volumetric heating from inside the material which makes this process efficient in processing time and heating rate (Indiarto and Rezaharsanto, 2020). As the food product itself acts as the electric conductor and the source of internal heat, this technology contributes to environmental sustainability by reducing gas emissions and water consumption during processing (Pereira *et al.*, 2020).

The effectiveness of OH lies in its combination of thermal and non-thermal effects such as electrical degradation, electroporation and electro-permeabilization. These mechanisms enhance the permeability within plant cell wall matrix, promoting the leakage of intracellular bioactive compounds (Capaldi *et al.*, 2024).

The ultrasound pretreatment prior to ohmic assisted hydro-distillation (OHH) shows some mechanical effect on cell walls causing the pouring out of essential oil from the glands due to the increased number of pores (Gavahian *et al.*, 2017). The combined action of cavitation and electroporation attributed to the release of active compounds trapped within plant cells by overcoming the inherent barriers developed by the cell walls. Zhang *et al.* (2022) reported that the Ultrasound Assisted Ohmic Hydro-Distillation (UAOHH) provides a higher extraction rate and energy savings. The combined process parameters are time, power, solvent-solid ratio and ohmic current. The increase in ultrasound power and electric current showed a positive correlation with the increase in yield. The antimicrobial activity of the sample was high enough to make the UAOHH a feasible method providing high quality of extract. Also, Jafari *et al.* (2022) investigated the influence of ultrasonic pretreatment prior to

OHH in anise seeds. The study concluded that UAOHH is a rapid, green, economical, and efficient extraction technique.

4.4 Ultrasound assisted sub-critical water extraction

Subcritical water extraction (SWE) is a novel water-based extraction in which hot water is maintained at high temperature below its critical point (100-374°C) and at high pressure (10-100 bar) to keep the water in liquid state during the extraction process. This alters the physicochemical properties of water, influencing its solvating characteristics. Effect of temperature is high enough to alter its polarity as well as viscosity, surface tension and density. The dielectric constant of water, which is approximately 80 at ambient conditions, decreases to around 30 at higher temperatures and behave similar to organic solvents such as ethanol or methanol (Kheirkhah *et al.*, 2019). So, by controlling high temperature and pressure, water turns to a suitable solvent for extracting polar to medium polar solutes (Co *et al.*, 2012). Maintaining elevated pressure keeps the water in its liquid form and allows better filling to matrix pores with water. It also ruptures the cell matrix causing enhanced mass transfer (Cvetanović *et al.*, 2018). The major advantages are the utilisation of water as solvent, faster extraction rate and enhanced extraction yield than conventional extraction.

While high temperatures can enhance extraction efficiency, they can also lead to the degradation of phenolic compounds. In contrast, it is also found that the partially degraded phenols enhance the antioxidant activity than their precursors (Co *et al.*, 2012). On the other hand, the total antioxidant activity depends on the nature of target substrate. The optimisation of temperature and pressure for each group of compounds is essential as they have different influence on extraction. Kheirkhah *et al.* (2019) reported that SWE recovered high value phenolic compounds from kiwifruit pomace with reduced amount of solvent and extraction time.

The major drawback faced by SWE is low mass transfer efficiency and uneven extraction with increase in loading quantity. With the aim of further enhancing the extraction yield, ultrasound can be introduced as a pretreatment (Guo *et al.*, 2021). The study inferred that combining ultrasound with SWE enhanced the quality of extract with higher antioxidant activity and efficiency.

4.5. Ultrasound assisted super-critical CO₂ extraction

Super critical fluid extraction is a novel alternative technology for natural compound extraction. Carbon dioxide (CO₂) with its moderate critical temperature (31.1°C) and pressure (7.3 MPa) and its Generally Recognized As Safe (GRAS) status, makes it the most commonly used fluid for extraction (Soares *et al.*, 2018). Super critical CO₂ (SCO₂) has combined gas and liquid like behaviour which enables the rapid and selective extraction due to their properties including high diffusivity and low viscosity. It produces high quality extracts with minimal thermal degradation (Tzima *et al.*, 2023). Further, extracts are readily precipitated without the need for solvent separation. However, the high equipment and operation cost, as well as lower extraction yield compared to the conventional methods, limits its application (Machado *et al.*, 2013). Furthermore, the addition of conventional solvents is required to enhance the extraction rate due to the low polarity of CO₂.

The addition of ultrasound as a pretreatment to SCO₂ extraction can address the limitations of low mass transfer and extraction rate in a sustainable way. The cavitation effect favours the solvent penetration, thereby enhancing mass transfer and extraction efficiency. Thus, the synergistic effect boosted the mass transfer efficiency and reduced the time of extraction, and consumption of CO₂ in the process (Yang *et al.*, 2016; Soares *et al.*, 2018).

Liu *et al.* (2020) exploited the synergistic effect of ultrasound and SCO₂ to enhance the extraction of cucurbitacin E from *Iberis amara* seeds. The loosened and porous structure created due to cavitation effect enhanced the mass transfer and resulted in higher extraction rate than the non-ultrasound treated one. In the extraction kinetics, the yield obtained through the hybrid technology was significantly higher than SCO₂ and conventional method at point of time. Similar findings were reported by Liu *et al.* (2023) in the extraction of compounds from *Cosmos sulphureus* seeds. The hybrid approach yielded higher extraction yields and resulted in extracts with improved physicochemical properties, antioxidant activity, and thermal stability. Thus, they concluded the fact that this hybrid approach is a high performance, sustainable technology for the extraction.

5. ADVANTAGES AND LIMITATIONS OF ULTRASOUND ASSISTED EXTRACTION

Ultrasound-assisted extraction (UAE) offers several advantages, including reduced extraction time, solvent consumption, and energy input. It enables lower temperature extraction, rapid energy transfer, high selectivity, increased extraction yield, improved mixing efficiency, and reduced thermal gradients. However, UAE also presents certain challenges. The mechanical energy input from

ultrasound can generate shock waves and free radicals (H and OH), which may lead to the degradation of heat-sensitive compounds. Since it is an electrical device, care must be taken by the operator (Zia *et al.*, 2022). Additionally, operational factors such as noise levels, temperature control, and limitations in handling high solid-to-liquid ratios must be carefully considered to optimize the extraction process (Capaldi *et al.*, 2024).

6. CONCLUSION

Ultrasound is a promising non thermal technology which has an immense potential in the extraction of bioactive compounds from diverse sources. It is a sustainable and environmentally friendly alternative to convention extraction methods. By leveraging the mechanical effects of cavitation, UAE enhances mass transfer, leading to improved extraction rates and yields while reducing energy consumption and solvent usage. The optimization of process parameters such as power, time, solvent-to-solid ratio, temperature, and frequency is crucial for maximizing the efficiency of the extraction process. The synergistic effect of ultrasound with other novel technologies significantly improves the overall extraction process by addressing the limitations of each individual approach. While these hybrid techniques may require higher initial investments and energy consumption, they offer significant benefits in terms of reduced processing time, improved product quality, and environmental sustainability. Future research should focus on optimizing the parameters of these combined techniques, exploring novel applications, and addressing the challenges associated with scaling up these processes for industrial production. By advancing the field of ultrasound-assisted extraction, we can develop more efficient, sustainable, and cost-effective methods for the recovery of valuable bioactive compounds from diverse sources.

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