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Ultrasonic assisted solvent extraction of bioactive compounds: A review

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Abstract
This review is about the Extraction of active chemical compounds from different sources using ultrasonically assisted solvent extraction procedure. It is one of the most important research areas for pharmaceutical and chemical industries. Traditional Solvent extraction methods were less efficient more costly because of excess use of solvents and associated with longer extraction times and lower yields.

Keywords: ultrasound, extraction, solvent

1. Introduction

The comprehensive analytical study of bioactive compounds from the dissimilar plant, fruits, herbal plants materials and by-products of plant origin mainly depend on the selection of proper extraction method [1, 2]. Extraction of the bioactive compounds from different plant materials and by-products of plant origin is the initiation step of any plant bioactive compounds analysis and has a direct impact on the final result and outcome of the entire extraction process. Extraction process sometimes is also called sample preparation techniques. Most of the time, non-trained research personnel Hennion *et al* 1998 [3], though two-third of effort is done by professionals. A study conducted by Majors 1999 [4] revealed that the most of researchers know the importance of sample preparation during any analysis of bioactive compounds. An Energing technique like chromatography and spectrometry make bioactive compound analysis simple and fast than conventional extraction methods but the end results largely depend on the extraction methods, input framework and exact nature of plant parts Poole *et al.* 1990 [5]. Elements influencing extraction method are properties of the plant part used extraction method, type of solvent used, temperature, pressure and time Hernandez *et al.* 2009 [6]. As a result in Advancement in technology and technical methods; pharmaceuticals, food additives even on natural pesticides sectors have become interested in bioactive molecules from natural sources. Characteristically, bioactive compounds remain intact with other molecules present in plants.

Bioactive compounds from different plant materials and by-products of plant origin can be identified and characterized. Extraction of bioactive compounds can be done by Non-conventional methods, which are more ecofriendly due to minimum use of synthetic and organic chemicals, less operational time, and better yield and quality of extract. To intensify total extract and selectivity of bioactive compounds from the dissimilar plant, fruits, herbal plants materials and by-products of plant origin, ultrasound, pulsed electric field, enzyme digestion, extrusion microwave heating, ohmic heating, supercritical fluids and accelerated solvents have been used as non-conventional methods. Traditional extraction methods, such as Soxhlet is believed to be as the reference procedure to compare the success of evolving extraction techniques.

2. Bioactive Compounds

Bioactive compounds include phytochemical that is found in the different plant, fruits, herbal plants and are efficient in regulating metabolic processes, resulting in the advancement of good health. In general, these compounds are mainly present in fruit, vegetables, and whole grains Carbonell-Capella *et al.* 2013 [7], Gil-Chavez *et al.* 2013 [8] and typically occur in small concentrations. These bioactive compounds show beneficial effects such as antioxidant properties, hampering or induction of enzymes, hampering of receptor action, and induction and hampering of gene expression Correia *et al.* 2012 [9]. Phytochemical can be considered as vastly heterogeneous group of compounds with varying chemical structures (hydrophilic/lipophilic), distribution in nature (specific to vegetable species/ubiquitous), difference in concentrations both in foods and in the human body, possible site of action, The potential for achieving results against oxidative species, and specificity and biological action Porrini and Riso 2008 [10]. Among them, polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organosulfur compounds constitute important groups in the human diet.Rising awareness among people about the health benefits of carotenoids has served fuel to global markets for these compounds with the result that in 2010 the market was an estimated $1.07 billion and is projected to top $1.2 billion by 2015 Global Industry Analysts, 2010 [11]. Carotenoids are present in fruits and vegetables as carotenes (unsatured hydrocarbons) and xanthophylls (oxygenated derivatives). Generally, the main carotenoids in vegetables are lutein, *β*-carotene, violaxanthin, and neoxanthin. In fruits, xanthophylls are commonly found in a maximum quantity. They are prone to isomerization and/or oxidation due to their unsaturation Hill and Johnson 2012 [12]. Only a very low quamtity of carotenoids has been reported to become bioaccesible Courraud *et al* 2013 [13]. In some fruits (such as mango, papaya) carotenoids are present in oil droplets in chromoplast and hydroxycarotenoids are chiefly esterified with fatty acids, being more easily extracted during digestion. The world market for polyphenols is high. For example, Leatherhead Food Research 2009 [13] estimates the current market is worth approximately $200 million. The majority of polyphenols extracted for sale as nutraceuticals or for use in functional foods come from either grapes, apples, olives or green tea and this is sent back in the studies that have been performed on optimising ex-traction by ultrasonic assistance. Phenolic compounds or polyphenols form a large group of chemical substances considered as secondary metabolites of plants. They have an aromatic ring and a benzene ring with one or more hydroxide groups, inclusive of phenolic acids (hydroxy-benzoic acids and hydroxy-trans-cinnamic acids), coumarins, flavonoids (flavones, flavonols, flavanones, flavanolols, flavanols, and anthocyanidins), isoflavonoids, lignans, stilbenes, and phenolic polymers (proanthocyanidins and hydrolyzable tanins) Springett, 2001 [14]. Phenolic compounds are inportant in the wine industry as it gives the characteristics colour and flavour in wine, and in the pharmaceutical industry for its benefits on human health Brenna *et al* 1998 [15]. Polyphenols are associated with reduced risk of cardiovascular disease by inhibiting in-vitro oxidation of lowdensity lipoproteins possess anti-ulcer, anti-mutagenic, anti-inflammatory activity and anti-carcinogenic properties Flamini, 2003 [16], Negro *et al*, 2003 [17], Bonilla *et al* 1998 [18], Palma & Taylor, 1999 [19]. Phenolic compounds include tannins and colour pigments, anthocyanins which present at a higher level in red grape marc compared with white grape marc and are more likely to be found on the grape seeds [14, 19]. Anthocyanins are a large class of water-soluble plant pigments based on the 2-phenylbenzophyrylium (flavylium) structure and there are more than 200 bioactive compounds in this category (IPCS, 2001). Anthocyanins are the main colour pigments in wild fruits and berries and mostly found in the sap of mature cells in the grape skin [14]**.** The grape skin consists of different pigments like di-glucosides, mono-glucoside, acylated monoglucosides and acylated di-glucosides of peonidin, malvidin, cyanidin, petunidin, and delphinidin. Anthocyanins content in grapes varies from 30–750 mg/100 g Birdle & Timberlake, 1997 [20]. The wide variation in the amount of these compounds is greatly dependent upon cultivar, season, growing conditions, the degree of ripeness, storage conditions as well as extraction procedures Cacace &Mazza, 2003 [21]. Many studies has been conducted on the potential to use ultrasound treatments for the extraction of pigments Cai, *et al*, 2003 [22].

3. Ultrasound assisted extraction

Ultrasound technology is not used as a solo method for commercial food products currently in the world market. However, ultrasound assisted technologies for product alteration or process advancement to exist Welti *et al* 2002 [23]. The aim of this paper to review assisted applications of ultrasound technology in the extraction of bioactive compounds.

i. Principles of Ultrasonics

The most applicable generation of ultrasound is carried out using the electrostrictive transformer principle. This is based on the elastic deformation of ferroelectric materials within a high frequency electrical field and is caused by the mutual attraction of the molecules polarized in the field Raichel, 2000 [24]. For polarisation of molecules, a high-frequency alternating current will be transmitted via two electrodes to the ferroelectrical material. Then, after conversion into mechanical oscillation, the sound waves will be transmitted to an amplifier, to the sound radiating sonotrode and finally to the treatment medium Dietrich Knorr *et al*, 2004.

ii. Extraction mechanisms

Ultrasounds are mechanic waves that result in elastic medium to spread over. The difference between sound and ultra- sounds is the frequency of the wave, sound waves are at human earring frequencies (from 16 Hz to 16–20 kHz) while ultrasounds have frequencies above human earring but below microwaves frequencies (from 20 kHz to 10 MHz). As a sound wave passes through an elastic medium, it induces a longitudinal displacement of particles. In fact, the source of the sound wave acts as a piston on the surface of the medium Mason *et al*, 1990 [25].

Resulting in a succession of compression and rarefaction phases into the medium. When the piston is in its opened position it induces a compression into the medium and when the piston is in its contracted (pull) position it creates a rarefaction phase. Every medium has an adverse molecular distance: below this critical value, the liquid remains intact, but above this distance, the liquid would break down and voids can be generated into the liquid. In the case of ultrasounds, if the rarefaction cycle is strong enough, the distance (d) between contiguous molecules can reach or even exceed the critical molecular distance of the liquid. The ultrasonic effect is responsible for createating voids into the medium by cavitation phenomenon. In fact theses, cavitation bubbles are able to grow during rarefaction phases and decrease in size during compression cycles. When the size of these bubbles attains a critical stage they collapse during a compression cycle and release large amounts of energy. The temperature and the pressure at the moment of collapse have been estimated to be up to 5000 K and 2000 atmospheres in an ultrasonic bath at room temperature. This creates hotspots that are able to accelerate dramatically the chemical reactivity into the medium. When these bubbles collapse onto the surface of a solid material, the high pressure and temperature released generate microjets directed towards the solid surface. These microjets are effective for the degreasing of metallic surfaces which is widely used for cleaning of materials. An- another application of microjets in the food industry is the extraction of vegetal compounds.

In Fig below a cavitation bubble can be generated close to the plant material surface (a), then during a compression

Cycle, this bubble collapse (b) and a microjet directed toward the plant matrix is created (b and c). The high pressure and temperature involved in this process will destroy the cell walls of the plant matrix and its content can be released into the medium (d). This is a considered to be an effective tool for extraction of bioactive compounds from natural products.

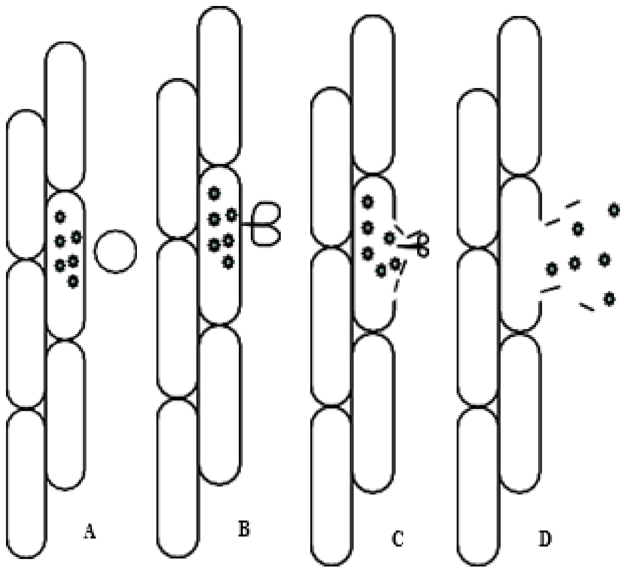

Fig 1

iii. Ultrasound assisted solvent extraction of bio active compounds

Ultrasound assisted extraction have also been mixed along with various conventional techniques as they are reported to increase the efficiency of a conventional system. In a solvent extraction unit, an ultrasound device is placed in a suitable position to increase the extraction efficiency Vinatoru *et al.* 1998 [26]. The benifits of Ultrasound assisted extraction are a reduction in extraction process time, energy and limited use of a solvent. Ultrasound energy for extraction also enhance mixing efficiency, high energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased production and removes process steps Chemat *et al*., 2008 [27]. Ultrasound assisted extraction is seemed to be an efficient extraction process for bioactive compound extraction from fruits, vegetables, herbal plants, medicinal plants etc. Rostagno *et al*. 2003 [28] showed extraction advancement of four isoflavones namely, genistin, daidzin, glycitin and malonyl genistin from soybean with the mix-stirring procedure using various extraction times and solvents. Researchers found that ultrasound can increase the efficiency of the extraction yield depending on solvent use. Herrera and Luque de Castro. 2004 [29] in there research on strawberries extracted phenolic compounds such as rutin, naringin, naringenin, quercetin, ellagic acid and using 0.8 s duty cycle for 30 s by flourishing semiautomatic procedure based on ultrasounds. Better recovery of chlorogenic acid from fresh leaves, fresh bark and dried bark of Eucommia ulmodies Oliv. By Li *et al*. 2005 [30] in his research using Ultrasonic at optimized condition (70% methanol, 20:1 solvent, sample ratio and 30 min time) than conventional extraction procedures. Yang and Zhang 2008 [31] used optimized sonication condition to obtain bioactive compounds called rutin and quercetin from Euonymus alatus (Thund.) Sieb and concluded that ultrasonic method had better extraction efficiency than conventional methods. Three alkaloids (vindoline, catharanthine and vinblastine) from Catharanthus roseus has been extracted using ionic Ionic liquid based UAE and is regarded as a very effective process [31]. Anthocyanins and phenolic compounds were extracted from grape peel using UAE and the extraction process was optimized with reference to solvent, extraction temperature and time Ghafoor *et al*., 2011, 2009 [32]. Phenolcarboxylic acids, carnosic acid and rosmarinic acid were extracted from Rosmarinus officinalis using Ionic liquid based UAE technique which was proved to have high efficiency and shorter extraction time than conventional extraction methods Zu *et al*. 2012 [33].

i. Industrial extraction application design

The use of ultrasound assisted extraction of bioactive compound from different plants fruits and herbal and medicinal plants has been reviewed by various authors. Recently, the construction of ultrasound processing equipment has improved to furnish industrially robust processing abilities. Authorizing design and operational features have included; (a) automated frequency examine to authorize maximum power delivery during fluctuation of processing conditions, (b) non-vibrational flanges on sonotrodes for construction of high-intensity inline flow-cells and (c) construction of radial and hybrid sonotrodes to give greater range in application design and product opportunities Chemat *et al*, 2010 [34]. Presently, 16 kWis the largest available single ultrasound flowcell, which can be configured in-series or in parallel modules. Industrial ultrasound manufactures within the last 2 years have promoted industrial processing capability for food extraction applications Hielscher, 2006 [35]. Many ultrasound reactor designs have been explained by Chisti 2003 [36] and Vinatoru 2001 [37], the latter specifically for industrial extraction of plant tissue. These included (a) stirred ultrasound horn (sonotrode) directly immersed into stirred bath or reactor, (b) stirred reactor with ultrasound coupled to the vessels walls and (c) recycling of product from stirred reactor through an external ultrasonic flow-cell. These arrangenments may provide both intermittent and continuous ultrasound exposure, from low intensity in a large capacity reactor (0.01 to 0.1 W/cm3) to high intensity (1 to 10 W/cm3) in an external flow-cell. Mixed frequency reactors have been shown to offer benifits with respect to process efficiency and energy distribution Vilkhu et ol,2007 [38].

Reactor geometries that are asymmetrical and polygons preferably with odd numbered sides using swept frequencies are also reported to be more effective [38]. Latest ultrasonic systems include automated frequency examining which adjusts operation of the system to the optimal frequency to ensure that maximum power is transmitted to the extraction vessel. The benefit of automated frequency scanning as opposed to a fixed frequency was demonstrated by Romdhane and Gourdan 2002 [39] where the former achieved a 32% increase in pyrethrine extraction and a 30% increase in power delivered to the product. The presence of a dispersed phase contributes to the ultrasound wave attenuation. The active sonication region in a reactor is restricted to a zone located at the surface of the probe.

Where it is not a disadvantage to extract oily materials as stable emulsions, ultrasound can be used to carry out aqueous extraction of oily materials with yields of the order of 50%.

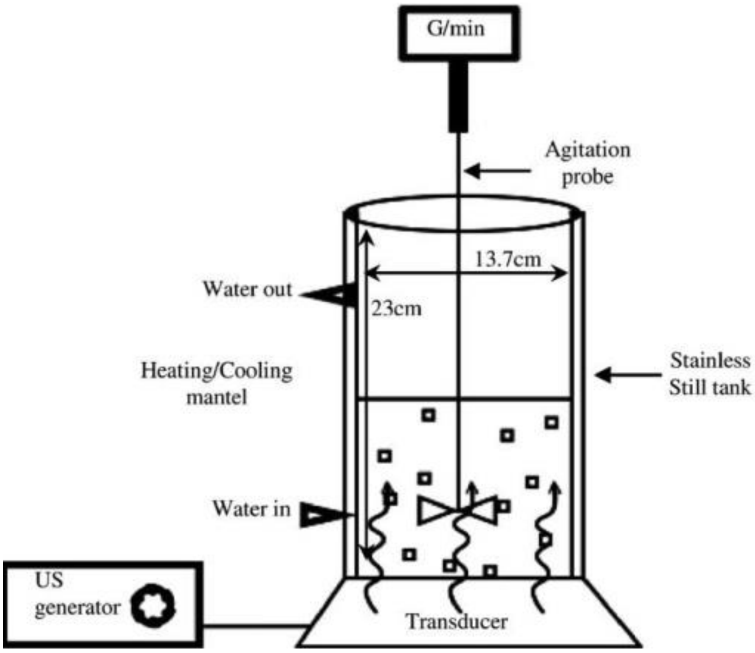

Fig 2: Sonication apparatus used for UAE

To improve efficiency the product to be used for extraction of bioactive compounds should be reduced to as smaller particle size as practical without denaturing the material to be extracted and commensurate with separation from the solvent post extraction. If this is done very high yields and extraction rates are possible with ultrasonic augmentation of the extraction process Balachandran *et al*., 2006 [40]. The proposed benefits of UAE for the food industry include, (a) overall, enhancement of extraction yield or rate, (b) enhancement of aqueous extraction processes where solvents cannot be used (juice concentrate processing), (c) providing the opportunity to use alternative (GRAS) solvents by improvement of their extraction performance, (d) enable sourcing/substitution of cheaper raw product sources (variety) while maintaining bioactive levels and (e) enhancing extraction of heat sensitive components under conditions which would otherwise have low or unacceptable yields.

ii. New opportunities for UAE in the food industry

There is a chance to capture new intellectual property in the area of ultrasound processing particularly where the technology can provide commercially attractive advantages and outcomes unique to ultrasound processing. Ultrasound has the unique capacity to both enhance extraction from substrates while simultaneously encapsulating the extracted substance with an encapsulating material in the extraction fluid by hydroxyl radical initiated covalent bonding and microsphere formation. To successfully accomplish this, the encapsulating material should have a higher reductive potential than the material being extracted and be relatively more hydrophobic. Preferably a mixed frequency ultrasound field is used, a relatively low frequency to facilitate extraction and a higher frequency under independent amplitude control to facilitate hydroxyl radical production for cross linking and microsphere formation. Proteins are put forward for the consideration of encapsulants as the sonochemistry and conditions favouring sphere development have been established. Vessel geometries, frequency combinations, and frequency modulation to achieve the desired outcomes on a large scale suitable for scale up to the industrial application would need to be explored and optimized.

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