A first-principles model for plant cell rupture in microwave-assisted extraction of bioactive compounds

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ABSTRACT

A first-ever theoretical model describing the cell rupture mechanism for microwave-assisted extraction (MAE) of bioactive compounds from plant samples is presented. This model incorporates the microwave heating of intracellular moisture within the plant cells using Lambert’s law. It then calculates the heating time and the intrinsic energy required to pressurize and stretch the plant cells until rupture using thermodynamic relations. The attainment of cell rupture is determined from elasticity theory for solids. The simulation requires the inputs of the effective shear modulus of the plant cell wall and the incident microwave irradiation flux entering the extraction mixture. The predicted cell rupture time by the intrinsic energy validated excellently against the optimum extraction time found in experiments. This model is generally applicable over a wide extraction parameters and microwave systems.

Keywords: theoretical model, microwave heating, cell rupture, extraction time, bioactive compounds

The published version is available on http://dx.doi.org/10.1016/j.jfoodeng.2016.05.017
1. Introduction

Microwave-assisted extraction (MAE) is among the efficient techniques to extract bioactive compounds from plants. It employs microwave to enhance the heating of the extraction solvent containing the plant materials. Generally, it requires shorter extraction time and lower volume of solvent compared to those using conventional heating such as Soxhlet extraction (Chan et al., 2011; Wang and Weller, 2006). Despite its superior performance, MAE is widely adopted only in the laboratory scale because the technique is difficult to scale up and its optimum operating condition is hard to gauge using different configurations. As such, the optimum operating conditions for MAE reported in the literature are applicable only for specific systems and on a restricted scale. These issues, which arise from a lack of fundamental understanding of the underlying physico-chemical processes, should be resolved before MAE can be fruitfully commercialized.

To this end, various optimization and modeling methods had been developed for optimization and scaling up of MAE. The modeling of MAE has been conventionally based on two-parametric empirical models such as film theory (Stankovic et al., 1994) and chemical kinetic equation (Rakotondramasy-Rabesiaka et al., 2007) to describe the dynamics in the washing and the diffusion steps of the extraction (Chan et al., 2014). The constants of the models rely heavily on experimental data, making them only useful in comparative studies such as to indicate the extraction kinetics at different operating conditions. Response surface methodology (RSM) and artificial neural network (ANN) models had also been applied to simulate and optimize the operating parameters of MAE (Sinha et al., 2013; Sinha et al., 2012). These models are able to simulate and optimize MAE using less experimental data compared to the two-parametric empirical models. However, screening of suitable range of operating parameters is needed to achieve reliable optimization results. Further, it is not obvious what those parameters should be on scale-up. Besides these efforts, modeling of MAE had been attempted using Maxwell’s equations for electromagnetism, energy and species balance equations via COMSOL Multiphysics™ software in the work (Chumnanpaisont et al., 2014). On top of simulating the extraction profile using empirical parameters, the model is able to capture the spatial distribution of electromagnetic wave and temperature profile in the MAE system. Recently, a few predictive mass transfer models (Chan et al., 2015a; Chan et al., 2013, 2015b) had been derived from two-parametric empirical model with the incorporation of energy-based parameters, namely the absorbed power density and
the absorbed energy density. The energy-based models is able to predict the optimum extraction time and extraction profile at various microwave powers, extraction scales, microwave system and heating modes (e.g. constant-power, intermittent-power and constant temperature). Despite the apparent success of the later developments, all parameters from the above-mentioned models have no physical meanings thus they are not suitable to be used for equipment design, process optimization and scale up. Hence, to complete the picture, theoretical modeling of MAE should consider the mechanism of rupturing plant cell as had been suggested by various works (Gujar et al., 2010; Yan et al., 2010; Zhang et al., 2013). To the best of our knowledge, there was no literature available on this topic.

For the first time the cell rupture mechanism of MAE is described using fundamental principles in this work. The ultimate goal is to investigate the intrinsic energy to rupture plant cells and to predict the optimum extraction time from the temporal-evolutions of the intracellular thermodynamics during MAE. This work presents the theoretical framework of the model in Section 2. Following that, a case study is elucidated in Section 3, followed by the results validated over a broad range of MAE conditions. Finally, conclusions to this study are given in Section 5.

2. Theoretical framework

This model was constructed based on the following premises. Plants samples contain a large number of parenchyma cells (Cutter, 1978) that store bioactive compounds. During MAE, microwave propagates within the extraction mixtures comprising of solvent and plant particles, the latter containing vacuoles with certain moisture content. Due to the favorable dielectric properties, the intracellular moisture of the plant particles absorbs a portion of the incident microwave energy, becomes heated and vaporized to generate internal pressure within the cell vacuoles. This causes the membrane surrounding the vacuoles to stretch and expand, eventually reaching the cell wall. Beyond that, the cell wall expands together. The membrane of the vacuole may or may not rupture, but this is immaterial as long as the cell wall remains intact. When the internal pressure exceeds the strength of the cell wall, the plant cell ruptures and the bioactive compounds are released into the surrounding solvent.
To account for the steps envisaged above, the cell rupture model needs to incorporate: (A) the pressure-volume relationship based on the mechanical properties of the parenchyma cell wall; (B) the average radiative heating power of intracellular moisture; and (C) the temporal-evolution of intracellular pressure, temperature and volume expansion, as summarized pictorially in Fig. 1. Details of each will be elaborated in the subsections below.

Fig. 1: Schematic of cell rupture model of MAE
2.1. Pressure-volume relationship of plant cell wall

The first part of the model requires the pressure-volume relationship of the cell wall, modelled based on elasticity principles for a polymer-like substance (Wu et al., 1985). Approximating the shape of a parenchyma cell wall as a thin-shelled hollow sphere, its pressure-volume relationship can be described as follows (Wu et al., 1985):

\[
P_{\text{int}} = P_{\text{atm}} + \gamma_0 \left[ \left( \frac{V_c}{V_{c0}} \right)^{-1/3} - \left( \frac{V_c}{V_{c0}} \right)^{-7/3} \right] 
\]

(1)

where \( P_{\text{int}} \) is the intracellular pressure. Since the extraction vessel is not pressurized, the surrounding pressure or the intercellular pressure is at the atmospheric pressure. \( V_c \) and \( V_{c0} \) are respectively the expanded and the initial volume of the plant cell. The lumped parameter \( \gamma_0 \) is defined as:

\[
\gamma_0 = \frac{2 t_{c0} G}{r_{c0}} 
\]

(2)

where \( t_{c0} \) and \( r_{c0} \) are the average thickness and radius of the cell wall in original condition, and \( G \) is the shear modulus. The parameter \( \gamma_0 \) which has the same units of but often a smaller magnitude than shear modulus, is known as the mechanical property constant of the plant cell wall. It can be interpreted as an effective shear modulus modified by the geometry.

The pressure-volume relationship is approximately linear at the beginning of the extension, by virtue of the form of the equation. The maximum of \( P_{\text{int}} \) occurs at \( V_c/V_{c0} = \sqrt[3]{7} \) regardless of the value of \( \gamma_0 \). The maximum point is also known as yield point whereby extension beyond this point can be interpreted as the plastic-flow state of cell wall (Li et al., 2010). While the elasticity principle could not predict \textit{a priori} whether the cell wall would rupture at, below or above this maximum pressure, for this pressure-volume model, the maximum pressure is assumed to be the cell rupture pressure. Upon rounding up, the cell rupture pressure is:

\[
P_{\text{rup}} \approx P_{\text{atm}} + 0.62 \gamma_0 
\]

(3)
2.2. Microwave heating analysis

As illustrated in Fig. 1(B), the second part of the model incorporates the dissipation of microwave power within the extraction mixture in a cylindrical vessel. In reality, this heterogeneous mixture is non-uniform, with more solid particles (plant sample fragments) near the bottom of the container, and there could potentially be convective movements of the particles during the heating. The dielectric properties of the solvent, solid matrix and any moisture trapped within are also different, giving significantly different heating response under microwave irradiation. Hence a rigorous way to account for the microwave heating of this mixture is not obvious.

However, at low solvent to solid ratio, only a thin layer of the mixture is reasonably clear, while the rest of the mixture is quite densely packed with solid particles. Moreover, based on experimental observation, the particles do not churn around significantly because the temperature gradient of extraction solvent, which promotes movement of particle due to convection current, is not significant under the volumetric heating of microwaves. As such, it is first assumed that the solid particles are uniformly distributed within the extraction solvent, and second that there is no relative movement of particles throughout the process. With this simplification, the number of distributed solid particles aligned along the radial, \( r \) and the vertical, \( z \) directions are respectively \( n \) and \( m \), related as below:

\[
\pi n^2 m = V_s \rho_t \\
\frac{n}{m} = \frac{R}{Z}
\]

where \( V_s \) is the volume of the solvent, \( R \) is the radius of the extraction vessel, \( Z \) is the level of solvent in the vessel and \( \rho_t \) is the total number of particles per unit volume of solvent, calculated as follows:

\[
\rho_t = \frac{SF}{SF} \cdot \frac{1}{V_p \rho_p}
\]

where \( SF \) is the solvent to solid ratio, \( V_p \) is the average volume of a particle and \( \rho_p \) is the intrinsic density of the particle.

With the spatial distribution of the particles fixed, it is logical to say that the particles nearer to the wall of the extraction vessel experience higher microwave flux than those in the interior due to
power absorption along the direction of propagation. For simplicity, the propagation of microwave is assumed to be primarily inwards from the side wall(s) of the extraction vessel since the side surface area is commonly greater than the top surface area. With this assumption, the wave propagates through a repeating pattern of a particle of diameter $D_p$, followed by a gap $L_s$ occupied by the solvent within the extraction solvent. If one further inspects each particle, a distance $L_p$ along its diameter is occupied by plant materials, while the remaining by moisture. Although in reality the moisture is not concentrated at any particular position within the leaf particle, the difference between a more finely distributed placement of the moisture and this lumped placement is expected to be small and does not warrant the additional complexity. Upon solving Eqs. (4), (5) and (6), the length of each medium in the domain can be obtained as follows:

Gap of the solvent medium, or the inter-particle spacing:

$$L_s = R - nD_p \left( \frac{L_n}{n+1} \right)$$

(7)

Thickness of the moisture within each particle:

$$L_w = \left( \frac{6}{\pi} V_w \right)^{1/3}$$

(8)

Thickness of the remaining plant material:

$$L_p = D_p - L_w$$

(9)

where $D_p$ is the equivalent spherical diameter of a plant particle and $V_w$ is the total volume of moisture in a plant particle. The configuration above facilitates approximation by a one dimensional propagation of microwave in the negative radial direction of the extraction vessel containing repeated multi-layered medium of solvent, plant materials and plant moisture arranged in sequence radially as depicted in Fig. 1(B).

Lambert’s law, which states that the transmitted microwave power flux entering the medium decreases exponentially with the distance from the surface of the medium (Ayappa et al., 1991), is used to analyze the microwave heating. It gives good approximation to the average radiative heating power of the mediums, without the need of Maxwell’s equations (Ayappa et al., 1991) since the exact distribution of power flux within the mediums is not vital in this study. A few additional assumptions are made: a) the components of the extraction mixture have constant dielectric properties, b) the reflection of microwave flux within the mixture is not significant due
to rough interfaces involved, c) the convective heat transfer across the medium is insignificant as plant materials generally have low thermal conductivities (Hays, 1975). In its simplest form, Lambert’s law expresses the variation of transmitted microwave flux, $I$, and the power absorbed per unit volume of the medium, $P_v$ with the distance of propagation, $x$ as follows (Ayappa et al., 1991):

$$I(x) = I_0 e^{-2\beta x}$$  \hspace{1cm} (10)

$$P_v(x) = -\frac{dI(x)}{dx} = 2\beta I_0 e^{-2\beta x}$$  \hspace{1cm} (11)

where $I_0$ is the incident power flux to the medium and $\beta$ is the attenuation constant of the medium. The dielectric property $\beta$ is given by (Ayappa et al., 1991):

$$\beta = \frac{2\pi f}{c} \sqrt{\frac{\kappa' - 1}{2}}$$ \hspace{1cm} (12)

where $c$ is the speed of light, $f$ is the frequency of microwave radiation, $\kappa'$ is the relative dielectric constant of the medium and $\kappa''$ is the relative dielectric loss of the medium. For microwave heating in a multi-layered medium, the corresponding expression for the transmitted power flux, $I_i$ and power absorbed per unit volume, $P_i^V$ at the $i^{th}$ medium are given as follows (Ayappa et al., 1991):

$$I_i(x) = I_0 \exp \left( -\sum_{j=1}^{i-1} 2\beta_j L_j \right) \exp \left( -2\beta_i (x - x_{i-1}) \right), \quad x_{i-1} \leq x \leq x_i$$ \hspace{1cm} (13)

$$P_i^V(x) = 2\beta_i I_i(x), \quad x_{i-1} \leq x \leq x_i$$ \hspace{1cm} (14)

In the above equations, $L_i$ and $\beta_i$ are the thickness and the attenuation constant of the $i^{th}$ medium, respectively, and $x_i$ is the propagated distance of microwave from the 1st medium to the $i^{th}$ medium. If $I_0$ is the transmitted power flux for a single medium (i.e. $i=1$), then Eqs. (13) and (14) would reduce to Eqs. (10) and (11). The volume-averaged absorbed power of the moisture medium per unit mass, $P_{w,av}$ can then be calculated as follows:

$$P_{w,av} = \frac{\int_V P_w^V(x) dV}{\rho_w \int_V dV}$$ \hspace{1cm} (15)

where $P_w^V(x)$ is the localized heating power of moisture medium ($i=w$) per unit volume, $V$ indicates the cylindrical volume of the extraction mixture and $\rho_w$ is the density of moisture.
It is vital to re-emphasize that the formulation above hinges on relatively stationary particles throughout the extraction mixture. At high solvent loading, the plant particles tend to move around within the extraction mixture, carried along by convection currents. Hence, the average heating power experienced by the moisture within the particles cannot be approximated by simple volume-average. A more sophisticated approach accounting for the hydrodynamics will be required. The approach proposed here is the simplest first attempt, which nevertheless is in line with the economic need to operate at low solvent loading.

2.3. Thermodynamic analysis of intracellular condition

If the microwave power absorbed by the plant particles as calculated above can be translated into intracellular pressure evolution over time, this will enable the cell expansion be predicted from the stress-strain relationship, thus completing the model. To build this last component of the model, it is hypothesized that there are three stages to change the thermodynamic state of the intracellular moisture from the ambient condition to the rupture condition, as illustrated in Fig. 1(C). For the same end points, Hess’s Law allows non-unique multiple paths to be chosen, here the most convenient and logical combination is chosen.

Initially, the cell membrane is shrunken as most of the moisture had been removed during the sample preparation stage such as drying and grinding. In this model, the moisture left within the membrane, or precisely within the vacuole, is responsible for the eventual cell rupture and its amount remains unchanged during the heating process. The latter is because the rehydration of dried plant cells by external solvent or water is insignificant (Deng et al., 2003; Lewicki, 1998; McMinn and Magee, 1997). The first heating stage involves heating of the intracellular moisture from the initial condition (state 1, 25 °C, 1 atm) to a saturated liquid (state 2, 100 °C, 1 atm). The time taken for the first heating stage, \( t_{1-2} \), is derived as follows:

\[
dQ_{1-2} = P_{w,av} dt_{1-2} = C_p dT
\]

\[
t_{1-2} = \int_{25^\circ C}^{100^\circ C} \frac{C_p}{P_{w,av}} dT
\]

where, \( Q_{1-2} \) is the heat absorbed per unit mass of moisture, \( C_p \) is the specific heat capacity of water, \( T \) is the intracellular temperature.
The second heating stage vaporizes the intracellular moisture at constant pressure to stretch the vacuole to its maximum volume, completely fill up the cavity within the cell wall (state 3, vapor-liquid equilibrium, 1 atm). As vapor volume at low pressure is much larger than the liquid volume, it is unlikely that all liquid water has been vaporized at this juncture, hence a vapor-liquid equilibrium should exist. Thermodynamic relations in Eqs. (18) and (19) can be used to describe this boiling stage:

\[ dQ_{2-3} = P_{w,av} dt_{2-3} = du + P_{\text{int}} dv \]  \hspace{1cm} (18)

\[ t_{2-3} = \frac{u_1 - u_2 + P_{\text{int}} (v_3 - v_2)}{P_{w,av}} \]  \hspace{1cm} (19)

where \( u \) and \( v \) are the specific internal energy and specific volume of moisture in the cell. The specific volume \( v_3 \) can be estimated from the specific volume of moisture in a plant particle:

\[ v_3 = \frac{\alpha \times V_p}{m_w} \]  \hspace{1cm} (20)

where \( \alpha \) is a ratio of total intracellular volume to volume of a plant particle and \( m_w \) is the mass of moisture in a plant particle. Specific internal energy \( u_3 \) can then be determined from the thermodynamic data of saturated steam at 1 atm based on \( v_3 \).

In the third and final heating stage, the vaporization of the intracellular moisture continues, further expanding the vacuole to stretch the cell wall until it ruptures at the maximum pressure (state 4, \( P_{\text{rup}}, V_c/V_{\text{co}} = \sqrt{7} \)). Similar thermodynamic relations as in Eq. (18) can be used to describe this heating process.

\[ dQ_{3-4} = P_{w,av} dt_{3-4} = du + \int_{v_3}^{v_4} P_{\text{int}} dv \]  \hspace{1cm} (21)

Changing the variable of integration in Eq. (21) to volume extension ratio, \( v_{\text{inc}} \), defined in terms of the volume of plant cell and specific volume:

\[ v_{\text{inc}} = \frac{V_c}{V_{\text{c},0}} = \frac{v}{v_3} \]  \hspace{1cm} (22)

we have

\[ P_{w,av} dt_{3-4} = du + v_3 \sqrt{7} \int_{1}^{v} P_{\text{int}} dv_{\text{inc}} \]  \hspace{1cm} (23)
Note that the $v_{inc}$ at stage 3 is unity as the cell is just about to expand whereas it is $\sqrt[3]{7}$ at rupture stage as given in Section 2.1 previously. A key difference here is that the intracellular pressure is no longer constant as it needs to overcome the rigidity of the cell wall. Substituting the pressure-volume relationship from Eq. (1) into Eq. (23) gives the heating time during this last stage:

$$t_{3-4} = \frac{u_4 - u_3 + v_3 \left[ P_{\text{amb}} \left( \sqrt[3]{7} - 1 \right) + \frac{3Y_0}{2} (7)^{3/3} - 1 \right]}{P_{w, av}}$$

(24)

The internal energy $u_4$ can be determined the thermodynamic data of saturated steam at $P_{rup}$ based on the specific volume $v_4 = \sqrt[3]{7}v_3$. The total time computed from Eqs. (17), (19) and (24) gives $t_{rup}$, the average heating time required to rupture the plant cells in the extraction mixture. Implicitly, $t_{rup}$ is regarded as the optimum extraction time of MAE. Combining $P_{w, av}$ and $t_{rup}$ gives the average energy required to rupture the plant cells per unit mass of moisture:

$$E_{rup} = P_{w, av} \times t_{rup}$$

(25)

### 3. Case study

The proposed model was constructed using MATLAB R2009b. The thermodynamic data for saturated steam used in simulating the intracellular condition was obtained from MATLAB File Exchange (Holmgren, 2006). In general, to apply the model, four key items must be known, namely: (a) the extraction solvent, (b) the solvent to solid ratio, (c) the mechanical properties of the parenchyma cells, and (d) the incident microwave power flux. Items (a) and (b) are decided by the user, while items (c) and (d) have to be measured experimentally. Commonly used solvents include water, ethanol (EtOH) and aqueous EtOH such as 85% v/v EtOH. The solvent to solid ratio under which the dense particle packing assumption might be reasonable ranges from 20-80 mL/g.

For item (c), the typical mechanical and physical properties of parenchyma cells found in fruits, leaves and stems are as follows: the shear modulus of cell wall, $G$ of 1-10 MPa (Gibson, 2012); the radius of cell, $r_{c0}$ of 5-25 μm (Gray et al., 1999; Lybeer et al., 2006; Nagy-Déri et al., 2011);
the thickness of the cell, $t_{c0}$ of 0.5-1.5 μm for fruits and leaves (Gray et al., 1999) and in the range of 1.5-3.0 μm for lignified parenchyma cells in the stem and the midrib (Lybeer et al., 2006). The mentioned properties are important for the determination of the parameter $\gamma_0$ using Eq. (10). To fulfil the thin-wall assumption, the model requires $t_{c0}/r_{c0} < 0.05$ (Juárez et al., 1999). Hence, the parameter $\gamma_0$ is selected in the range of 0.04-2.00 MPa. On the other hand, the volume ratio of total intracellular space to a plant particle, $\alpha$ was taken as 0.9 since the vacuole of plant cell can fill as much as 90% of the intracellular space (Wiebe, 1978).

Item (d) requires the incident power flux $I_0$. The amount of microwave power absorbed in the system, $P_{abs}$, can be found from the temperature rise over time of a given volume of blank extraction solvent. $P_{abs}$ can also be expressed in terms of $I_0$ by taking the volume integral of $P_\nu$ in Eq. (11) as follows:

$$
P_{abs} = \frac{m_s C_{p,s} \Delta T_s}{t_H} = \int_0^Z \int_0^{2\pi} \int_0^R 2\beta I_0 e^{-2\beta r} r dr d\theta dz
$$

(26)

where $m_s$ is the mass of solvent, $C_{p,s}$ is the heat capacity of solvent, $T_s$ is the temperature of solvent and $t_H$ is the heating duration. Equating both:

$$
I_0 = \frac{\beta m_s C_{p,s} \Delta T_s}{t_H \pi Z (1 - 2Br e^{-2\beta r} - e^{-2\beta R})}
$$

(27)

The performance of the model to predict the optimum extraction time was validated with the experimental values in the MAE of bioactive compounds from cocoa (*Theobroma cacao* L.) leaves reported in (Chan et al., 2013). The MAE was conducted at constant heating power mode using a conventional microwave oven (2.45 GHz). The input parameters for this validation study are tabulated in Table 1.
Table 1: Input parameters of cell rupture model in validation study

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Moisture of the plant sample</td>
<td>5% (w/w) dry basis</td>
</tr>
<tr>
<td>2. Volume of a plant particle, $V_p$</td>
<td>0.0452 mm$^3$</td>
</tr>
<tr>
<td>3. Intrinsic density of the plant sample, $\rho_p^a$</td>
<td>2169 mg/cm$^3$</td>
</tr>
<tr>
<td>4. Radius of cylindrical extraction vessel, $R$</td>
<td>38.5 mm</td>
</tr>
<tr>
<td><strong>Dielectric properties of mediums</strong>$^b$</td>
<td>$\kappa'$ $\kappa''$ $\beta$ (m$^{-1}$)</td>
</tr>
<tr>
<td>5. Water</td>
<td>79.30 11.70 33.62</td>
</tr>
<tr>
<td>6. 85% EtOH</td>
<td>20.00 13.00 71.23</td>
</tr>
<tr>
<td>7. EtOH</td>
<td>6.80 7.00 62.42</td>
</tr>
<tr>
<td>8. Plant material</td>
<td>2.15 0.09 1.57</td>
</tr>
<tr>
<td><strong>Extraction conditions</strong></td>
<td>Experimental optimum time (min)$^c$</td>
</tr>
<tr>
<td>9. 100 W, 100 mL of 85% EtOH, 50 mL/g</td>
<td>33.3</td>
</tr>
<tr>
<td>10. 200 W, 100 mL of 85% EtOH, 50 mL/g</td>
<td>11.6</td>
</tr>
<tr>
<td>11. 300 W, 100 mL of 85% EtOH, 50 mL/g</td>
<td>5.4</td>
</tr>
<tr>
<td>12. 600 W, 100 mL of 85% EtOH, 50 mL/g</td>
<td>2.2</td>
</tr>
<tr>
<td>13. 200 W, 200 mL of 85% EtOH, 50 mL/g</td>
<td>20</td>
</tr>
<tr>
<td>14. 250 W, 250 mL of 85% EtOH, 50 mL/g</td>
<td>13.5</td>
</tr>
<tr>
<td>15. 300 W, 300 mL of 85% EtOH, 50 mL/g</td>
<td>11.9</td>
</tr>
<tr>
<td>16. 100 W, 100 mL of 85% EtOH, 20 mL/g</td>
<td>30</td>
</tr>
<tr>
<td>17. 100 W, 100 mL of 85% EtOH, 80 mL/g</td>
<td>27</td>
</tr>
</tbody>
</table>

$^a$ measured by pycnometer

$^b$ properties taken from Navarrete et al. (2011) and Sato and Buchner (2004)

$^c$ Values obtained from Chan et al. (2013)

$^d$ calculated using on Eq. (27)

4. Results and discussion

4.1. Microwave heating characteristics in the extraction mixture

Generally, a medium with higher attenuation constant $\beta$ such as 85% EtOH is capable of absorbing more microwave energy, as simulated in Fig. 2(a). The plant material medium is inert to microwave thus its interaction with microwave is negligible. As a result of power absorption, less microwave flux is transmitted through the medium. Note that the local transmitted power flux is continuous at the medium interfaces, whereas the local absorbed power shows discontinuity as the
power absorption depends on the $\beta$ value of medium according to Eq. (14). As microwave propagates through the mediums in the extraction mixture, the local absorbed power of solvent, plant materials and moisture decreases exponentially, approaching zero at the center of the extraction vessel as illustrated in Fig. 2(b). The volume-average absorbed power in the figure denotes the average heating power experienced by the mediums in the extraction vessel during the MAE. This average heating power is consistent regardless of the direction of microwave propagation either in $-r$, $-z$ or both $-r$ and $-z$ directions since it is determined based on the total power absorbed in the extraction system calibrated using Eq. (26). Apparently, propagation direction of microwave affects the incoming flux $I_0$ but not the amount of power flux attenuated or absorbed in the extraction system.

The effects of parameters affecting the volume-average heating power per unit mass of moisture medium, $P_{w.av}$, namely the incident power flux, the type of extraction solvent and the solvent to solid ratio are plotted in Fig. 3. The result shows that $P_{w.av}$ increases linearly with the microwave irradiation flux ($I_0$), while the gradient rises with decreasing values of the attenuation constant $\beta$ of the extraction solvent. The effect of the range of solvent to solid ratio investigated, represented by the error bars, is secondary. Interestingly, an extraction solvent with 85% ethanol gives the least amount of power absorbed by moisture in the plant particles at any incident power. This is because the addition of water in ethanol enhances the microwave absorption of the solvent medium via the formation of hydrogen-bonded complexes and the presence of cooperative microwave dielectric relaxation processes (Sato and Buchner, 2004). Although water facilitates faster cell rupture due to the higher $P_{w.av}$, its application in MAE depends on a number of other considerations. First of all is the solubility of bioactive compounds in water. Second is the potential degradation of bioactive compounds such as flavonoids (Biesaga, 2011) at the normal boiling point of water, which then requires advanced intermittent power and temperature-controlled heating modes as mitigation. Thus from a practical perspective, ethanol or aqueous ethanol is preferable in a simple MAE system with constant-power heating mode as the boiling point is milder (e.g. 78 °C for EtOH), besides it is often a better solvent for organic materials.
Fig. 2: (a) Simulated transmitted power flux and absorbed power per unit volume in solvent, plant material and moisture mediums. (b) Simulated local absorbed power in the extraction mixture. MAE conditions: 85% EtOH, 300 W, 50 mL/g and 2 g of sample.
Fig. 3: Simulated average heating power of plant moisture per unit mass during MAE at various incident power flux and extraction solvent. Error bars indicate the range of variations at a solvent to solid ratio of 20-80 mL/g.

In general, Fig. 3 is applicable to different microwave systems at other heating powers since $I_0$ can be calibrated experimentally. It is valid for any size of the extraction vessel as the volume has been accounted for in determining the average heating power. Extension to vessels of other shapes requires caution as they might not be axisymmetric as assumed in the current model. We would also caution the use of Fig. 3 to higher solvent to solid ratios as there is uncertainty whether the plant particles would move about significantly.
4.2. Optimum extraction time based on intracellular thermodynamics

Considering the intracellular moisture is heated by the average heating power $P_{w,av}$, the temporal evolution of its temperature, pressure and volume expansion for a typical case is depicted in Fig. 4. During stage 1, the temperature of the intracellular moisture is increased to the boiling point at atmospheric pressure. Then, some moisture vaporizes to push the cell membrane to the cell wall in stage 2. In Stage 3, the vaporization continues, causing the cell wall to expand, resulting in a rise of temperature and pressure beyond the normal boiling point, finally leading to cell rupture. The simulated rupture time agrees well with the experimental optimum extraction time (5.4 min) of MAE of bioactive compounds from cocoa leaves under identical conditions. Hence, it suggests that the average time required to heat the intracellular moisture from its initial state to the rupture state can be considered as the optimum extraction time of MAE.

![Diagram](image)

Fig. 4: Intracellular thermodynamics of a plant cell during MAE. MAE conditions: 85% v/v EtOH, 300 W, 50 mL/g and 2 g of sample. Normalized experimental yield was obtained from work (Chan et al., 2013). Model input parameters: $\gamma_0 = 0.7$ MPa; $I_0 = 0.1$ W/mm$^2$. $T_{int}$ is the intracellular temperature, $P_{int}$ is the intracellular pressure and $v_{inc}$ is the volume extension ratio of plant cell.
Further validation of the model at various extraction conditions as in Table 1 was conducted at the selected value of $\gamma_0 = 0.7$ MPa. The predicted cell rupture time ($t_{rup}$) at these conditions are compared with the experimental values of the optimum extraction time in Fig. 5. The match is remarkable. The results shows that the optimum time prediction is accurate regardless of microwave power level, solvent loading, solvent to solid ratio and type of microwave system. However, 30% overshoot occurs when predicting the optimum extraction time at a high solvent to solid ratio of 80 mL/g, most likely attributed to significant particle movement, allowing particles deeper in the vessel to be heated more than expected as they move towards the wall. This hints that the model generally tends to over-predict the required duration, making it a conservative model. More importantly, Fig. 5 strongly supports cell rupture as the dominant mechanism in the MAE of bioactive compounds from plants, with $t_{rup}$ being essentially the optimum extraction time. It further suggests that the conventional mass transfer mechanism, which is the diffusion of bioactive compounds due to concentration gradient, is not significant in MAE.

![Fig. 5: Comparison between the simulated cell rupture time at $\gamma_0 = 0.7$ MPa and the experimental optimum extraction time (Chan et al., 2013) of MAE of bioactive compounds from cocoa leaves. The solvent used was 85% (v/v) EtOH and the solvent to solid ratio was 50 mL/g unless otherwise specified.](image)
4.3. Energy for cell rupture

The total energy absorbed per unit mass by the intracellular moisture to rupture plant cells is given simply by $E_{rup} = t_{rup} \times P_{w,av}$. This shows up as the slope of the lines in Fig. 6. For a given value of $\gamma_0$, it seems that $E_{rup}$ is a constant. In other words, the total energy required to rupture a given type of plant cell is fixed, a higher absorbed power will give a shorter rupture time, and vice versa. This suggests that the total energy absorbed could be related to the change in a thermodynamic state, which is enthalpy, of the cellular contents. The simulated total energy absorbed for cocoa leaves (5% moisture, $\gamma_0 = 0.7$ MPa) in Fig. 6 was compared with the enthalpy change of intracellular moisture at the start (liquid, 25 °C, 1 atm) and at the end (saturated liquid-vapor, pressure $P_{rup} \approx P_{atm} + 0.62\gamma_0 \approx 0.535$ MPa, specific volume $v_4 = \sqrt[7]{\alpha V_p / m_w} = 0.022$ m$^3$/kg) of MAE. The simulated total energy absorbed (666.7 J/g) is indeed close to the enthalpy change of the moisture (671.6 J/g). The difference is attributable to the variable pressure between stage 2 and stage 3, which makes the heating path-dependent and not strictly equal to the enthalpy change. Further, the minute difference indicates that the change in the internal energy dominates over the expansion work, which is expected for vapors with large temperature changes. As such, the enthalpy change of the cellular contents can be related approximately as:

$$\Delta H_{1-4} \approx P_{w,av} \times t_{rup}$$

(28)

Here $\Delta H_{1-4}$ depends only on the initial condition and the incipient rupture condition of the plant cells, the latter of which is characterized by the mechanical property ($\gamma_0$) and the moisture content of the plant cells. Note that Fig. 6 was simulated based on plant cells with 5% moisture content. Conceptually, plant cells with higher moisture content will have smaller intracellular space, thus require less time and hence energy to pressurize the space to rupture. Nonetheless, the effect of higher moisture content in reducing the enthalpy change is not significant compared to the effect of $\gamma_0$ and is thus not presented in this study. This observation is consistent with the relatively small contribution of expansion work to the enthalpy change as alluded above.
Since $E_{rup}$ characterizes the energy required to break the plant cells for a specific plant, it has potential as a design parameter and could be used to establish the basic principle of design for MAE of bioactive compounds from plants. For instance, $E_{rup}$ could be useful in designing industrial microwave extractors, especially the configuration of the extraction vessel and the power output for the desired heating power and energy. Besides, it is possible to extract various plant samples simultaneously in MAE as the plant samples would be ruptured once their $E_{rup}$ are met.

To optimize and scale up the MAE, $E_{rup}$ enables the prediction of the optimum extraction time ($t_{rup}$) at any extraction conditions such as extraction solvent, heating power, solvent loading, solvent to solid ratio and microwave system. Overall, the intrinsic parameter $E_{rup}$ proposed in this work gives great operational flexibility and adaptability for different types of plant extractions to the MAE.
5. Conclusion
This theoretical model proves that the cell rupture mechanism is dominant in MAE of bioactive compounds from plants. The energy required to rupture plant cells \( E_{rup} \) by the intracellular moisture depends primarily on the mechanical property of plant cell wall \( \gamma_0 \), such that the value of \( E_{rup} \) is high for small plant cells with thick cell walls of high shear modulus. Prediction of the optimum extraction time \( t_{rup} \) by \( E_{rup} \) is quite accurate, based on the absorbed power of the intracellular moisture \( P_{w,av} \) calibrated by this model. This model is generally applicable over a wide variety of extraction parameters, namely extraction solvent, sample particle size, microwave power, solvent loading and solvent to solid ratio, with no restriction on the type of microwave system. Nevertheless, this model is valid at low solvent to solid ratio (i.e. < 50 mL/g) as the effect of the convective movement of plant particles during heating in not considered. This aspect should be explored in future refinements of the cell rupture model for MAE.

Acknowledgements
This work was financially supported through Fundamental Research Grant Scheme (FRGS: FP076-2015A) and University of Malaya Research Grant (UMRG: RP002A-13AET).

Nomenclature

Symbols

- \( c \) speed of light (ms\(^{-1}\))
- \( C_p \) specific heat capacity of water (J/gK)
- \( C_{p,s} \) specific heat capacity of solvent (J/gK)
- \( D_p \) equivalent diameter of a plant particle (mm)
- \( E_{rup} \) average energy required to rupture the plant cells per unit mass of moisture (J/g)
- \( f \) frequency of microwave radiation (GHz)
- \( G \) shear modulus (MPa)
- \( I \) transmitted microwave flux (W/mm\(^2\))
- \( I_0 \) incident power flux to the medium (W/mm\(^2\))
- \( I_i \) transmitted power flux in the \( i \)th medium (W/mm\(^2\))
\( L_i \)  \( L_{ip} \)  \( L_s \)  \( L_w \)  
characteristic length of the \( i \)th medium (mm)  
characteristic length of plant material medium (mm)  
characteristic length of solvent medium (mm)  
length of moisture medium (mm)  

\( m \)  \( m_s \)  \( m_w \)  
number of distributed sample plant particles along the height (particles)  
mass of solvent (g)  
mass of moisture in a plant particle (g)  

\( n \)  \( n_s \)  
number of distributed sample plant particles along the radial direction (particles)  

\( P_{abs} \)  \( P_{int} \)  \( P_{i}^V \)  \( P_{rup} \)  \( P_{atm} \)  \( P^w \)  \( P_{w,av} \)  \( Q \)  
absorbed power of solvent (W)  
intracellular pressure (MPa)  
power absorbed per unit volume of the \( i \)th medium (W/mm\(^3\))  
intracellular pressure to rupture the plant cell (MPa)  
大气压 (MPa)  
power absorbed per unit volume of medium (W/mm\(^3\))  
volume-averaged absorbed power per unit mass of the moisture medium (W/g)  
heat absorbed per unit mass of moisture (J/g)  

\( r \)  \( R \)  \( r_c \)  \( r_{c0} \)  
radial direction (mm)  
radius of extraction vessel (mm)  
radius of stretched plant cell (μm)  
radius of original plant cell (μm)  

\( SF \)  \( T \)  \( T_s \)  
solvent to solid ratio (mL/g)  
intracellular temperature (°C)  
solvent temperature (°C)  

\( t \)  \( t_{H} \)  \( t_{c0} \)  \( t_{rup} \)  
heating time (s)  
heating time in calibration experiment to find incoming flux \( I_0 \) (s)  
thickness of original cell wall (μm)  
heating time required to rupture plant cells or optimum extraction time (s)  

\( u \)  \( V \)  \( v \)  \( V_c \)  
specific internal energy of intracellular moisture (J/g)  
cylindrical volume of the modeling domain (mm\(^3\))  
specific volume of intracellular moisture (mm\(^3\)/g)  
expanded volume of the plant cell (μm\(^3\))
\( V_{c0} \) original volume of the plant cell (\( \mu m^3 \))

\( v_{inc} \) volume extension ratio of plant cell (dimensionless)

\( V_p \) volume of a grounded plant particle (\( cm^3 \))

\( V_s \) volume of solvent (\( mm^3 \))

\( V_w \) total volume of moisture in a grounded plant particle (\( mm^3 \))

\( x \) distance of propagation (mm)

\( x_i \) propagated distance of microwave from the 1\(^{st}\) medium to the \( i^{th}\) medium (mm)

\( z \) height (mm)

\( Z \) level of solvent in the extraction vessel (mm)

**Greek letters**

\( \alpha \) ratio of total intracellular volume to volume of a plant particle (dimensionless)

\( \beta \) attenuation constant of a medium (\( mm^{-1}\))

\( \beta_i \) attenuation constant of the \( i^{th}\) medium (\( mm^{-1}\))

\( \gamma_0 \) mechanical property constant of plant cell (MPa)

\( \theta \) angular direction (degree)

\( \Delta H \) enthalpy change of intracellular moisture (\( J/g \))

\( \kappa' \) relative dielectric constant of a medium (dimensionless)

\( \kappa'' \) relative dielectric loss of a medium (dimensionless)

\( \rho_p \) intrinsic density of the plant particle (\( g/cm^3 \))

\( \rho_t \) total number of particles per unit solvent volume (particles/\( cm^3 \))

\( \rho_w \) density of water (\( g/mm^3 \))
References


