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EXTRACTION OF 6-GINGEROL IN SUBCRITICAL WATER EXTRACTION- A CORRELATION OF DEGRADATION RATE AND EFFECTIVE DIFFUSION COEFFICIENT

A. N. ILIA ANISA[1,*], YOSHIO IWAI[2], NOOR AZIAN MORAD[3]

[1]School of Engineering, Faculty of Innovation & Technology,
Taylor's University, Subang Jaya, 47500, Selangor, Malaysia
[2] Department of Chemical Engineering, Kyushu University,
744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[3]Malaysia-Japan International Institute of Technology,
Universiti Teknologi Malaysia, 54000 Kuala Lumpur, Malaysia
*Corresponding Author: IliaAnisa.Aris@taylors.edu.my

Abstract

In this study, an empirical model to describe the extraction mechanism of 6-gingerol in subcritical water extraction was conducted by introducing the degradation rate (*kd*) and effective diffusion coefficient (*De*). The extraction of 6-gingerol was conducted using an Accelerated Solvent Extractor (ASE 350) at the respective times of 1, 3, 5, 7, 9, 11, 15, and 30 min at various temperatures ranging from 100°C to 180°C. The ASE 350 is an automated unit to extract 6-gingerol under a subcritical condition using water as a solvent. About 5.0 g of the dried and ground ginger was inserted into an extraction cell at the desired times and temperatures. The pressure remained constant at approximately 1500 psi to maintain the water in a liquid state. The extraction was performed automatically once the extraction cell was loaded into the oven. The extracted sample was flushed into the collection vial after completing the prescribed extraction time before further analysing in HPLC. The mass transfer was performed based on the concentration of the 6-gingerol obtained from the extraction process. The domination of the mass transfer coefficient was identified from the dimensionless Biot number (*Bi*). The *Bi* obtained was within 159.57 to 59.15 as the temperature increased from 100°C to 180°C. Results showed that *De* and *kd* increased from 1.93×10^{-8} to 3.38×10^{-8} m^2/min, and 0.0915 to 0.2511 min^{-1}, respectively, from 100 to 180°C.

Keywords: Biot number, Degradation rate, Effective diffusion coefficient, Mass transfer, Subcritical water extraction.

1. Introduction

The extraction of bioactive compounds sought great attention due to the application of bioactive compounds as secondary metabolites. Bioactive compounds play a vital role in many applications, such as medicines, food additives in the food industry, and cosmetics. The primary constituent of ginger is lipophilic rhizome extracts, which yielded potential activities. The most abundant compound in the ginger matrix is 6-gingerol. It possesses various biological activities for medicine, including anti-cancer, anti-inflammatory, and anti-mastitis [1]. The selection of the appropriate extraction method is essential to optimize the yield of the compounds. The conventional extraction method is time-consuming and requires a higher volume of toxic solvent; thus, the waste from extracted solvent should be treated to purify the bioactive compounds. In contrast, the non-conventional methods such as microwave, ultrasonic, and subcritical water extraction have lower extraction times and use more green solvents without concern of the waste treatment. The extraction method is in conjunction with selecting the appropriate solvent to alter the properties, thus increasing the solubility and mass transfer into the plant matrix or the sample. The 'green' extraction method compromises energy consumption, sustainability concerning the safe solvent, and the high quality of the extracts [2, 3].

Subcritical water extraction utilizes water as a solvent to alter the properties of water below the critical temperature. Water is introduced as a solvent in the extraction process, which is highly appropriate from an environmental point of view since it is the greenest possible solvent replacing the organic solvents. In addition, water has advantages such as a cheap, non-toxic to health, and safe for consumption. One of the vital characteristics of water is that its cohesive energy is primarily controlled by the hydrogen bonds formed between the water molecules. The hydrogen bonds are weakened at high temperatures, thus changing the polarity. The changing of polarity in subcritical water extraction by increasing the temperature reflects in its dielectric constant. The dielectric constant of water at 25^{o}C and ambient pressure is 78.5, which is suitable for extracting most polar compounds [4]. The mass transfer of compounds using subcritical water extraction comprises the diffusion and convection processes [5]. The subcritical water allows the solute's diffusivity, thus increasing the solute-solvent interaction. In addition, the convection of the solute-solute to the solvent also increases in the subcritical water extraction.

Understanding the kinetic studies of the extraction could predict the optimal operating conditions required before exposing the bioactive compounds to degradation. Optimizing the extraction process requires many experimental designs, specifically at a wide range of temperatures; it is not practical and economical. Fundamental knowledge of the mechanism extraction is crucial in upscaling the extraction of compounds to a higher capacity. The complexity of bioactive compound extraction contributes to the presence and localization in the microstructural of the plant matrix. Delocalization of the compounds to the solvent can be explained by the diffusivity and mass transfer of the compounds [6]. Four steps are generally involved in describing the whole extraction process [7]. The first step (I) is transporting the water or bulk solvent into the pores of the plant matrix. The second (II) one is the desorption of the compounds within the plant matrix from its location. The less bound compounds in the plant matrix, the compounds can be easily desorbed from their location. The third (III) step is transporting the compounds to the stagnant film surrounding the plant matrix. The

last (IV) step is removing or eluting compounds to water or bulky solvent across the stagnant film. The kinetic mechanism responsible for this step is the concentration gradient between the compounds in the plant matrix and compounds in the bulk solvent. The overall extraction steps are depicted in Fig. 1.

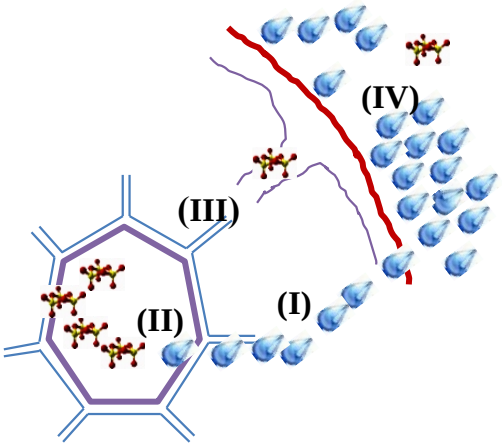

Fig. 1. Overview of the bioactive compounds extract from plant matrix.

The dimensionless Biot, *Bi* number can define the domination of mass transfer of the extracted compounds from the plant matrix. A lower value of *Bi* (<0.1) indicates that the external mass transfer is dominated. Meanwhile, *Bi* is higher than 1 is a domination of the internal mass transfer [8]. There are limited studies concerning the fundamental knowledge of extracting bioactive compounds from the plant matrix using subcritical water extraction. It includes the properties such as diffusivity, mass transfer coefficient, and solubility of the bioactive compounds. In addition, since the subcritical water extraction operates at elevated temperature and saturation pressure, the degradation rate should be considered. The limitation was when dealing with the thermo labile or heat-sensitive. The 6-gingerol is a bioactive compound sensitive to heat and tends to degrade. Thus, this is a challenge for the utilization of this technique that has to be overcome. This paper identified a correlation between mass transfer domination and degradation rate in the extraction of 6-gingerol using subcritical water extraction.

2. Materials and Methods

2.1. Material

Dried and ground ginger was obtained from a local supplier in Pahang, Malaysia. The sample was sieved with a size range between 0.60 to 2.36 mm using a mechanical shaker (BS 410/1986) to ensure a uniform sample size. The sample, with a size of 1.59 mm was selected for the extraction process. The 6-gingerol bioactive compounds was purchased from Chromadex (Irvine, CA). HPLC grade for methanol (ACS, Houston, USA), acetonitrile (Fisher, Loughborough, UK), and ultrapure water (Barnstead, USA) with resistivity >18.2 MΩ was used in HPLC analysis to determine the concentration of 6-gingerol.

2.2. Physical properties of ginger

2.2.1. Bulk density

The bulk density, ρ_b was determined using a method of ASTM C29/C29M-09. An empty 25 ml volumetric flask was weighed prior inserting the dried and ground ginger into the volumetric flask. Water was then inserted into the volumetric flask until reaching the calibrated mark. Eq. (1) was applied to calculate the ρ_b.

$$\rho_b = \frac{W_1 - W_2}{V} \tag{1}$$

2.2.2. Solid density

The solid density, ρ_s was measured using a pycnometer (AccuPyc II 1340, US). The density was determined by measuring the pressure change of helium in a calibrated volume.

2.2.3. Porosity and Tortuosity

The porosity, ε, was calculated from the correlation between the bulk and solid densities (g/cm^3) as shown in Eq. (2).

$$\varepsilon = 1 - \frac{\rho_b}{\rho_s} \tag{2}$$

Meanwhile, the tortuosity, $\tau = \boldsymbol{\varepsilon^{-0.5}}$

2.3. Extraction of 6-gingerol

The schematic diagram of Accelerated Solvent Extraction, ASE 350 (Dionex, Malaysia) is shown in Fig. 2. About 5.0 g of dried and ground ginger were weighed and mixed with 1.1 g of diatomic earth (P/N 062819, Dionex) in a 100 ml stainless steel extraction cell with a cellulose filter (P/N 056780, Dionex) placed at the bottom before loading the extraction cell into the ASE 350.

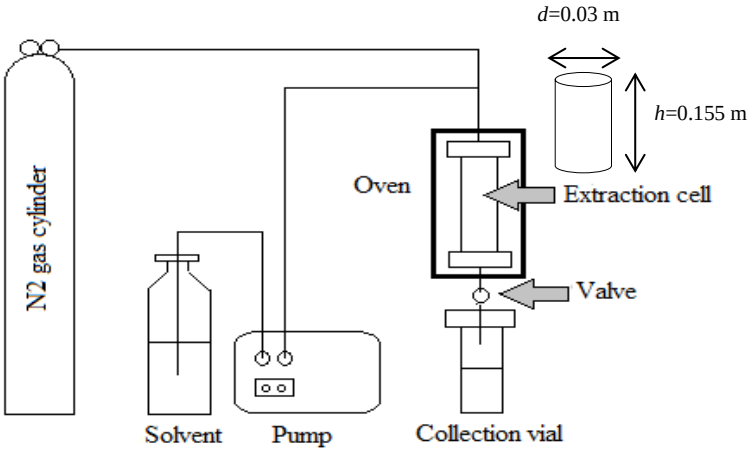

Fig. 2. Schematic diagram and cell extractor dimension of ASE 350.

In the ASE 350, there were four stages automatically operated as follows, (i) filling the cell, (ii) heating, (iii) static, and (iv) flushing. The solvent was filled between 0.5 to 1.0 min once the extraction cell was loaded into the oven. Then, followed in the heating stage, the extraction cell was heated until it reached the desired temperature, and pressure was maintained at 10.3 MPa (1500 psi). Thus, the solvent remained in the liquid state under subcritical conditions. Next, the extraction cell was kept in the oven at the prescribed extraction time. Finally, the extracted sample was flushed into a 250 ml collection vial using compressed nitrogen before further analysis in the HPLC.

2.4. HPLC analysis

The concentration of 6-gingerol was quantified using a High-Performance Liquid Chromatography, HPLC (e2695 Waters, USA), with a photodiode array detector. The 6-gingerol compounds was purchased from Chromadex, USA, and was separated in a C18 column (symmetry ®) of 5.0 μm particle size and dimensions of 150 mm × 4.6 mm. The mobile phases of HPLC analysis were methanol and acetonitrile HPLC grade (≥99.9%). The analysis was performed using Empower™ software embedded in the HPLC.

2.5. Kinetic and Thermodynamics Studies

2.5.1. Biot number

The dimensionless Biot number, *Bi* was calculated to identify the domination of mass transfer. The *Bi* < 10, external mass transfer is dominant, and *Bi*>10, internal mass transfer is dominant [8].

$$Bi = \frac{k_f D_p}{2D_e} \tag{3}$$

2.5.2. External mass transfer coefficient

The external mass transfer coefficient, k_f was calculated using the following Eqs. (4) and (5), as shown [9]:-

$$k_f = \frac{ShD}{D_P} \tag{4}$$

$$Sh = 2 + 0.95\ (Re)^{0.53} + (Sc)^{1/3} \tag{5}$$

where *Sh*, *Re*, and *Sc* are Sherwood, Reynold, and Schmidt dimensionless numbers, respectively. The *Re* and *Sc* numbers are calculated using the following equations.

$$Re = \frac{D_P v \rho}{\mu} \tag{6}$$

$$Sc = \frac{\mu}{\rho D} \tag{7}$$

The calculation of ρ and μ [10]

$$\rho\ (\frac{kg}{m^3}) = 858.03 + 1.2128T(K) - \ 0.0025T(K)^2 \tag{8}$$

$$\mu\ (\frac{kg}{m.s}) = \exp\ (-10.2 + (280970/T\ (K)^2)) \tag{9}$$

2.5.3. Effective diffusion coefficient

The effective diffusion coefficient, *De*, of 6-gingerol was determined using a correlation with diffusion coefficient, *D* [11]:

$$D_e = \frac{\varepsilon}{\tau} D \tag{10}$$

The diffusion coefficient, *D*, was predicted through mean squared displacement in molecular dynamics simulation using the software of SCIGRESS ME Compact 2.0 (Fujitsu Ltd, Japan). The values of *D* were applied to calculate the *De* [12].

2.5.4. Degradation rate

The degradation rate, k_d was determined from the non-linear regression of the empirical model. The empirical model was adapted from Fattah et al. [13]. The overall extraction of 6-gingerol from the ginger matrix involved extraction and degradation. From the *Bi* number, the dominant mass transfer in the extraction process would describe the extraction stage. Meanwhile, for the degradation part, the stage was defined by the degradation rate. The value of degradation rate, k_d was determined from experimental data using the non-linear regression model curve-fitting and solved in Matlab software (Matlab® version 8.1, The Mathworks Inc., Natick, USA).

$$C_w = \frac{C_o k_{ext}}{k_d - k_{ext}} [e^{-k_{ext}t} - e^{-k_d t}] \tag{11}$$

The C_0 is the initial concentration of 6-gingerol.

2.5.5. Initial concentration, *Co*

A Soxhlet apparatus [14] was set up to extract bioactive compounds from 70 g of sample with 280 ml of ethanol for 8 hrs. The extracted sample was evaporated in a rotary evaporator (Buchi R-205, Switzerland) until all the ethanol was removed by having a constant weight of the sample. The yield obtained was taken as an optimum yield, and the samples extracted from ASE 350 were compared with this benchmark yield.

3. Results and Discussion

The physical properties of the sample (ginger matrix) are listed in Table 1. The scale of effective diffusivity or diffusion coefficient depends on the tortuosity. The diffusivity of a solid porous medium deviated from the straight line due to the tortuosity [15]. The physical properties were determined to be applied in identifying the effective diffusion coefficient as stated in Eq. (10)

The effective diffusivity describes the ability of compounds was extracted from the plant matrix. This ability depends on the porosity of the wall plant matrix, as studied comprehensively by Li et al. [16] on the micronutrient for the different types of plant matrix walls. The dominant mass transfer of 6-gingerol extracted in subcritical water extraction was identified from the dimensionless Biot, *Bi* number. At 100, 120, 140, 160 and 180 °C, the *Bi* were 159.57, 118.93, 97.73, 71.26, and 59.15, respectively. The values of *Bi* decreased as increasing the temperature. It

showed that the effective diffusivity significantly dominated the internal mass transfer at the higher temperature. This is due to the surface's intensification changes showing an insignificant effect in extracting the bioactive compounds [8]. Setford et al. [17] stated that another factor contributing to the higher *Bi* in the extraction of malvidin-3-glucoside from fresh grapes was rigorous mixing during maceration and fermentation processes. Further details on the effective diffusivity at different temperature is tabulated in Table 2.

Table 1. Physical properties of ginger matrix.

Property	Value
Solid density, ρ_s	1.263 kg/m^3
Bulk density, ρ_b	0.789 kg/m^3
Porosity, ε	0.376
Tortuosity, τ	1.632

Table 2. Effective diffusivity for 6-gingerol extracts in subcritical water extraction.

Temperature (°C)	Effective diffusion coefficient, *De*(m^2/s)
100	1.93×10^{-8}
120	2.21×10^{-8}
140	2.36×10^{-8}
160	2.99×10^{-8}
180	3.38×10^{-8}

From Table 2, the effective diffusion coefficient was temperature-dependent. At 100°C, the *De* was 1.93×10^{-8} m^3/s, increasing almost two folds at 180°C. The increase of *De* is related to the higher solubility of subcritical water in the ginger matrix. At high temperatures, solvent viscosity decreased, thus enhancing mass transfer or solvent to extract the compounds. The increased diffusivity with temperature was in agreement with other studies on extracting bioactive compounds [18, 19]. They obtained the effective diffusivity from Fick's law by correlating it to the yield in the cycle of extraction time. Reche et al. [8] correlated the effective diffusivity with the Arrhenius equation from the values of the pre-exponential factor (2.41×10^{-5} m^2/s) and activation energy of 12.7 kJ/(mol.K) proposed mathematical modeling of the bioactive compounds extracted from artichoke by-products.

The external mass transfer based on degradation rate was predicted in this study using a non-linear regression as stated in Eq. (11). The initial concentration, C_o of 6-gingerol obtained in the ginger matrix was 56.846 ± 0.545 mg/g, and the method described previously in Section 2.5.5. The concentration of 6-gingerol with respect to temperature (100 to 180°C) was performed in a batch process for 1, 3, 5, 7, 9, 11, 15, and 30 min, as shown in Fig. 3. The curve-fitting model consists of two stages, (i) the extraction of 6-gingerol from the ginger matrix to solvent and (ii) the degradation of 6-gingerol in hot compressed water. The empirical model was chosen due to the assumption that the extraction and degradation of the bioactive compounds within the plant matrix occurred simultaneously.

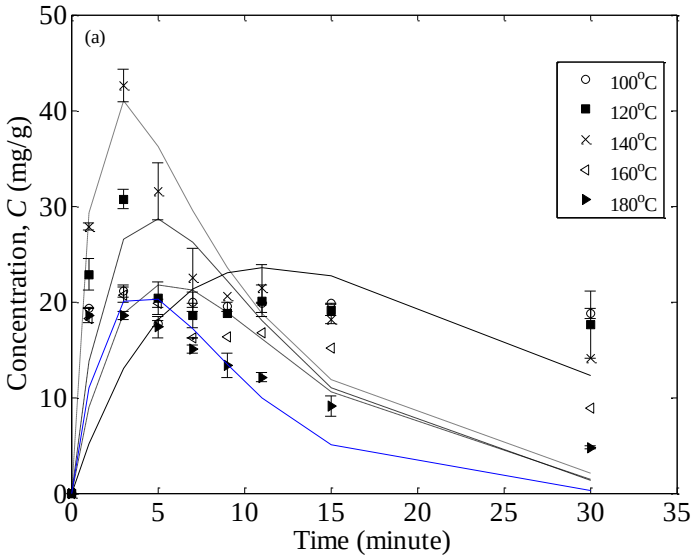

Fig. 3. The experimental fitting of the empirical model in the batch process in ACE 360. The solid line (—) for 100°C, a dashed line (- - -) for 120°C, a dotted line (.....) for 140°C, a dashed and dotted line (-·-) for 160°C, and a blue line (—) for 180°C.

From the figure, the curve-fitting model was well-fitted with the experimental data at high temperature. The adjusted-R^2 were 0.8386, 0.6473, and 0.7700 at 140, 160 and 180°C, respectively. However, at 100 and 120°C, the adjusted-R^2 were 0.4343 and 0.4036 with degradation rates, k_d of 0.0768 and 0.0914 min^{-1}, respectively. The lower values of adjusted-R^2 at 100 and 120°C due to the degradation were not obvious as at higher temperatures. This was observed at 30 and 3 mins, the concentration of 6-gingerol was almost similar. Overall, the extraction of 6-gingerol at different temperatures in subcritical water extraction showed two phases of mechanisms. Firstly, the effective diffusion coefficient contributed by diffusivity was observed. In the second stage, decreasing the concentration of 6-gingerol after reaching the optimum was described by the degradation. This trend was initiated by Fattah et al. [13], who concluded in their findings investigating the effect of degradation of oil recovery in subcritical water extraction that the degradation rate was increased significantly until certain values reached a steady state.

Finally, the correlation between effective diffusivity and degradation rate was identified and plotted in Fig. 4. Both effective diffusivity and degradation rate were defined by the internal mass transfer rate, in which at the higher temperature, the diffusivity of 6-gingerol from the plant matrix was increased, and at the same time, the compounds was exposed to the degradation. A slow increase of the degradation rate at 140°C indicated the optimal yield of 6-gingerol was obtained. However, a drastic increase in the degradation rate is observed at above 140°C due to exposure to elevated temperatures. The 6-gingerol is a thermal labile compound that tends to convert to 6-shogaol during thermal cracking [20, 21]. Ko et al. [20] found that the amount of 6-gingerol extracted from peel ginger in subcritical water extraction decreased at 130 °C, but a drastic increase of 6-shagoal was obtained.

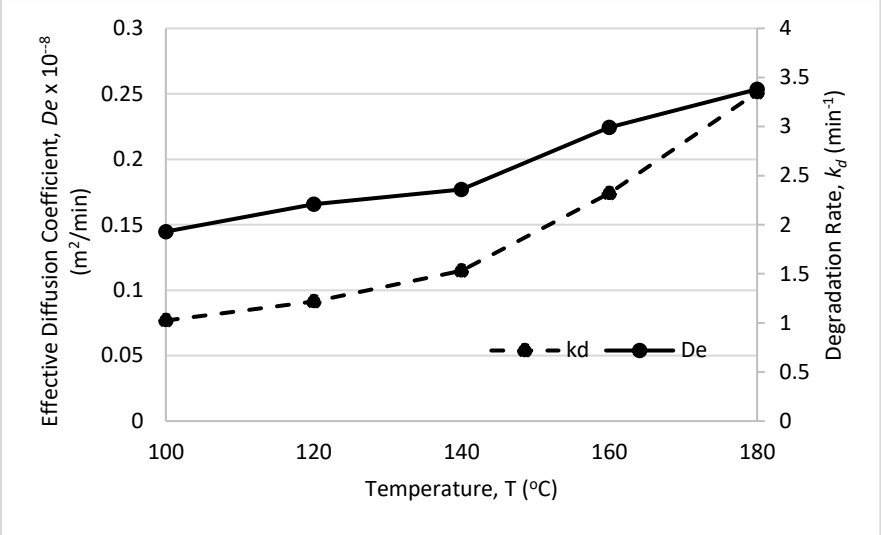

Fig. 4. Correlation between effective diffusion coefficient and degradation rate.

4. Conclusions

The extraction of 6-gingerol in the subcritical water extraction was dominated by the internal mass transfer, which further comprehensive mass transfer on effective diffusion coefficient was conducted. The effective diffusion coefficient was temperature-dependent, 100 °C to 180 °C increased from 1.93 to 3.38×10^{-8} m^2/s, respectively. A rapid increase in the degradation rate at 140°C indicated the 6-gingerol was exposed to the degradation. As a result, a proportional correlation was observed between the effective diffusion coefficient and degradation rate for the extraction of 6-gingerol in subcritical water extraction.

Nomenclatures

Bi	Dimensionless Biot number
Co	Concentration of 6-gingerol in initial, mg/g
Cw	Concentration of 6-gingerol in solvent, mg/g
D_e	Effective diffusion coefficient, m^2/s
D_p	Ginger diameter, m
k_d	Degradation rate, min^{-1}
k_{ext}	Extraction rate, min^{-1}
k_f	External mass transfer coefficient, m/s
Re	Reynold number
T	Temperature, K
t	Time, min
Sc	Schmidt number
Sh	Sherwood number
V	Volume of water occupied in the volumetric flask, mL
v	Velocity, m/s
W_1	Weight of ginger matrix in a 25 mL volumetric flask, g

W_2	Weight of empty 25 mL volumetric flask, g
Greek Symbols	
ρ_b	Bulk density, kg/m^3
ρ_s	Solid density, kg/m^3
ε	Porosity
μ	Kinematic viscosity, kg/(m.s)
τ	Tortuosity

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