Kinetics of Flavonoid Extraction from Dandelion Grass using Pressurized Liquid Extraction

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Abstract. This study investigates the kinetics of flavonoid extraction from dandelion using pressurized liquid extraction technology. 60% ethanol was employed as the solvent, and flavonoids were extracted at 80, 90, and 100°C under 0.3 MPa. A second-order kinetic model was utilized to predict the relationship between flavonoid concentration and temperature. Results indicate a good fit between the kinetic model and experimental data (R>0.95). The extraction kinetic equation is described as: Ct=t/ {1/[exp (127.92-45650 /T)]+t/(0.3247T- 113.13)}, which can be utilized for optimization and control of the flavonoid extraction process from dandelion grass.

Keywords: Dandelion Flavonoids; Pressurized Liquid Extraction; Dynamics.

1. Introduction

Dandelion, a well-known traditional Chinese medicinal herb, refers to the whole plant of Taraxacum mongolicum Hand.-Mazz. It is commonly used in traditional medicine for its efficacy in clearing heat, detoxification, and promoting diuresis and alleviating edema. Flavonoids are considered as the main pharmacologically active compounds in dandelion. Recent research has revealed various biological activities of dandelion flavonoids, including antioxidant, anti-inflammatory[1], and anticancer properties[2]. Its antioxidant effects help eliminate free radicals, alleviate cellular oxidative stress, and contribute to anti-aging and prevention of chronic diseases. Additionally, its anti-inflammatory properties can alleviate inflammation and pain, making it useful in treating upper respiratory tract infections, mumps, and acute tonsillitis[3]. Despite the numerous benefits of dandelion flavonoids, their widespread clinical application has been limited due to low extraction yields and high production costs associated with traditional extraction methods.

Recognizing the significant clinical value of dandelion flavonoids, there is a growing need to overcome the drawbacks of traditional extraction methods such as low extraction yields and time-consuming procedures. In this context, the emergence of pressurized extraction technology addresses these challenges. Pressurized extraction technology operates by maintaining solvents in liquid state at temperatures above their boiling points by increasing pressure within a closed vessel[4]. Elevated extraction temperatures effectively enhance solvent solubility and selectivity, thereby increasing the extraction efficiency of active ingredients[5,6]. Compared to traditional extraction methods, pressurized extraction technology not only saves costs and improves extraction efficiency but also facilitates industrial automation of extraction processes[7]. It has been widely applied in the extraction of natural products, medicinal herbs, pharmaceuticals and environmental pollutants analysis[8]. While the use of pressurized extraction technology for extracting bioactive compounds has gained attention, research efforts have primarily focused on laboratory-scale process, with limited studies on mass transfer kinetics during extraction processes. In-depth understanding of extraction

mechanisms and kinetic characteristics can provide valuable insights for engineering scale-up and production optimization. Many scholars have observed various extraction processes and proposed kinetic models to rationalize mass transfer processes in extraction operations[9-13]. However, due to the diversity of natural product tissue structures and the complexity of extraction processes, there is currently no universally applicable model to comprehensively reflect the kinetics of natural product extraction processes. Among these kinetic models, the "second-order" kinetic model proposed by Adamou Harouna-Oumarou et al.[14] is considered the most suitable for explaining the mass transfer mechanisms in solid-liquid extraction processes. This study suggests that extraction essentially involves the process of transferring effective components (i.e., solutes) from the solid phase to the liquid phase. The process can be divided into two consecutive stages: (1) Fresh solvent penetrates and dissolves the solute within the solid phase, with most of the extraction occurring in this stage; (2) then the solute diffuses from the interior of the solid phase to the external solvent phase, and this stage is slow and rate-limiting. This represents a typical second-order process, which can be explained by plotting the relationship between t/Ct and time. During the extraction process, according to Fick's law, the outward diffusion of soluble components depends on the concentration difference between the solid and liquid phases. Therefore, the three main influencing parameters that accelerate the extraction process are temperature, solid-liquid ratio, and particle size. Most literature primarily examines the influence of extraction temperature and establishes the relationship between the extraction rate constant and temperature, thereby deriving the extraction kinetics equation at any temperature. This facilitates adjustment of extraction parameters in up-scale production processes to achieve optimal extraction results.

This study attempts to simulate the mass transfer process of flavonoid extraction from dandelion using a "second-order" kinetic model based on Fick's law. The aim is to provide valuable theoretical basis for the optimization of flavonoid extraction from dandelion and even the extraction process of traditional Chinese medicinal herb components.

2. Material and Methods

2.1 Samples and Chemicals

Dandelion grass was purchased from Dequandang Pharmacy, Qingdao. Rutin (99%) was obtained from the China National Institutes for Food and Drug Control. Methanol (99.9%) and ethanol (99.8%) were obtained from Laiyang Economic and Technological Development Zone Fine Chemical Factory. Sodium nitrate, aluminum nitrate, and sodium hydroxide were purchased from Tianjin Guangcheng Chemical Reagent Co., Ltd.

High-speed universal grinder was obtained from Tianjin Test Instrument Co., Ltd. UV-Visible spectrophotometer was obtained from Beijing Liberty Instrument Co., Ltd.

2.2 Extraction Procedure

Pressurized liquid extraction was conducted in a custom-built extractor in our laboratory. 20 g of sample was ground using a high-speed universal grinder and placed into the extractor, along with 1200 mL of 60% ethanol solution. Extraction was initiated by heating the extractor to the desired temperature (80, 90, 100°C) and beginning extraction at 0.3 MPa. At time points of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 90 minutes, 10 mL of extraction solution was collected from the nozzle and stored at -20°C prior to analysis.

The content of flavonoids in dandelion was determined using a UV-Visible spectrophotometer, and the average of three replicates was calculated.

2.3 Quantitative Determination of Total Flavonoids

Flavonoids in the extract were quantitatively analyzed using a UV-Visible spectrophotometer. The relationship between absorbance (A) at 510 nm and the mass concentration (c) of rutin (μg·mL-1)

was determined to be: A = 12.759c - 0.0373, R = 0.9995. The linear range was 5.14 μg·mL-1 to 18.60 μg·mL-1.

Prior to quantitative analysis, all obtained extracts were filtered and adjusted to a specific volume with 60% ethanol. After adding a coloring reagent, scanning was conducted from 400 to 700 nm. Following validation of the maximum absorption wavelength at 510 nm, flavonoid concentration was quantitatively determined based on the calibration curve of the standard compound.

2.4 The Kinetic Model

In this process, flavonoids in dandelion were extracted using pressurized solvent extraction, which is a typical solid-liquid phase diffusion mass transfer process. A second-order extraction model is the most suitable for solid-liquid extraction processes. The kinetic model of PLE can be fitted to experimental data, and the extraction rate constant can be determined.

According to the second-order kinetic model, the extraction rate of dandelion flavonoids can be expressed as:

$$dCt/dt = k(Cs-Ct)^2 \quad (1)$$

When t = 0~t, C t = 0~C t, integrate and rearrange equation (1), the linear expression of Ct and t can be found as:

$$t/Ct = 1/kCs^2+t/Cs \quad (2)$$

Where: k is the extraction rate constant, mL·mg-1·min-1; C s is the mass concentration of flavonoids in the saturated solution, mg·mL-1; C t is the mass concentration of flavonoids in the solution at time t, mg·mL-1.

Transforming equation (2), the extraction rate h can be expressed as:

$$h = Ct/t = 1/ [(1/kCs^2) + (t/Cs)] \quad (3)$$

When t=0, the initial extraction rate h_0 can be expressed as:

$$h_0 = kCs^2 \quad (4)$$

Then the relationship between the mass concentration of dandelion flavonoids and the initial extraction rate at any time can be expressed as:

$$Ct = t/ [(1/h_0) + (t/Cs)] \quad (5)$$

The initial extraction rate h_0 and saturated mass concentration Cs can be obtained by plotting t/Ct against t.

3. Results and Discussion

3.1 Effect of Temperature on the Extraction Kinetics of Dandelion Flavonoids

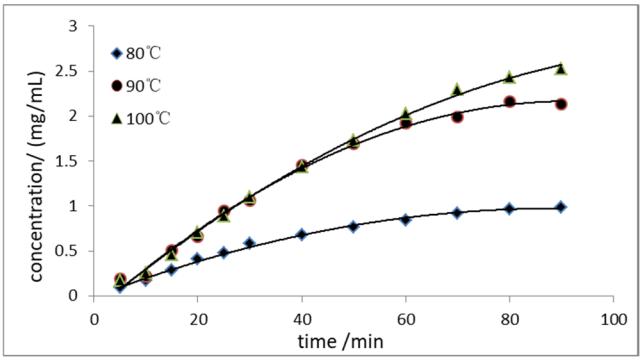


Fig 1. The effect of temperature on the concentration of dandelion flavonoid

From Fig. 1, it can be observed that at 80, 90, and 100°C, the mass concentration of flavonoids rapidly increased during the initial stage of extraction. After 80 minutes, the extraction reached equilibrium at 80 and 90°C, while the extraction rate remained fast at 100°C. Fig. 1 also shows that the flavonoid concentration at 80°C was consistently lower than at 90 and 100°C at any time point.

This may be because the boiling point of 60% ethanol is 81°C, and at the extraction temperature of 80°C, the extraction solvent has not reached the subcritical state, thus not exhibiting the advantages of subcritical fluid. With increasing temperature, both initial and final concentrations showed an increasing trend. In fact, the extraction process can be considered as a desorption process, and thus the Allenius law can explain this phenomenon.

Medicinal plants are immersed in the extraction solvent, and extraction begins when the extraction temperature reaches the preset temperature. Therefore, each curve in Fig. 1 does not pass through the origin. It can be seen from Fig. 1 that due to the short immersion and extraction time of the medicinal materials, the differences in the initial data recorded are not significant. Therefore, the recording time of the initial data was delayed to 25 minutes in Fig. 2.

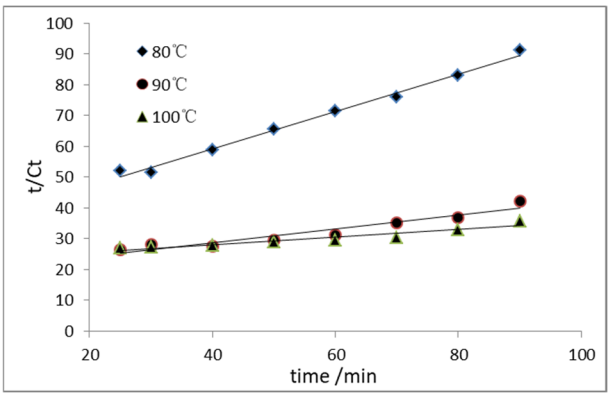


Fig 2. Extraction kinetics of dandelion flavonoid at various temperatures

The influence of temperature on solid-liquid extraction was studied with temperatures ranging from 80 to 100 ℃. The plot of t/Ct versus t (Fig. 2) shows that the extraction of dandelion flavonoids can be represented in a linear form according to the second-order model (R>0.95). The fitting results of the data under three temperature conditions according to the established kinetic equation are shown in Table 1.

Table 1. Kinetic Models of Dandelion Flavonoid Extraction

No.	*T* (℃)	Fitting equation	*Cs*(mg·mL^{-1})	h_0 (mg·mL^{-1}·min^{-1})	*k* (mL·mg^{-1}·min^{-1})	R
1	80	y = 0.609x + 34.749	1.6420	0.21	0.0776	0.9956
2	90	y = 0.2238x + 19.664	4.4683	20.27	1.0153	0.9577
3	100	y = 0.1229x + 23.112	8.1367	189.65	2.8646	0.9544

As expected, the initial extraction rate h_0, extraction capacity (Cs), and second-order rate constant k increase with increasing temperature in Table 1. Additionally, the initial extraction rate at 90°C is approximately 10 times that at 80°C, indicating a significant increase in the extraction rate of flavonoids when the extraction temperature exceeding the boiling point of the solvent (at subcritical conditions). This may be attributed to the significantly increased solubility and selectivity of the subcritical fluid compared to the solvent at ambient condition, resulting in a significant increase in the saturation concentration of the extract; moreover, the viscosity of the subcritical fluid significantly decreases, allowing it to better overcome mass transfer resistance, thereby significantly increasing the initial extraction rate. When the extraction temperature is set below the boiling point of the extraction solvent, there is little effect on the saturation concentration and initial extraction rate of the extract due to minimal changes in the physical and chemical properties of the solvent.

3.2 Extract Kinetic Equations

According to Equation (5), at a certain temperature, the extraction concentration Ct at time t is related to h_0 and Cs, which in turn are related to the extraction temperature.

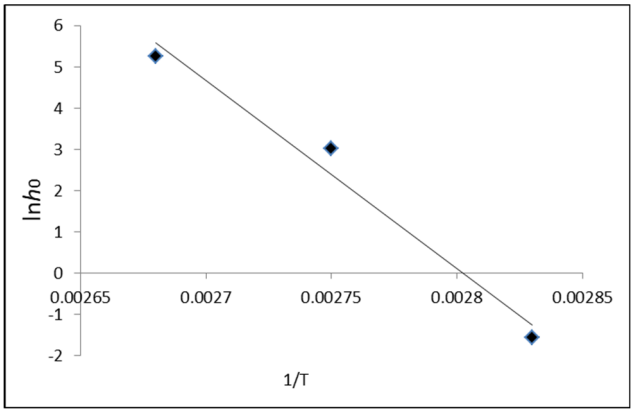


Fig 3. Linear Regression Plot of $\ln h_0$ ~1/T

By plotting $\ln h_0$ against 1/T (Fig. 3), the relationship between the initial extraction rate and temperature can be expressed as: $h_0 = \exp(127.92-45650/T)$, R = 0.9756.

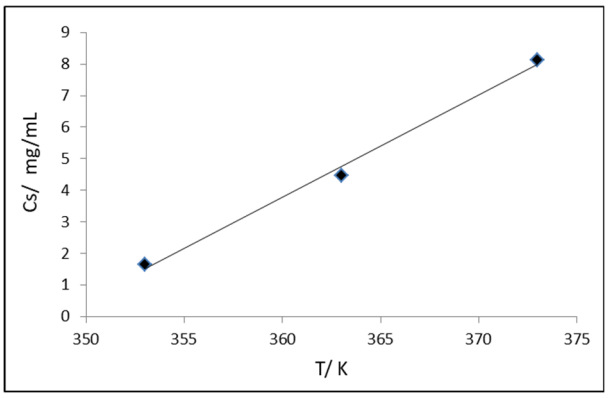


Fig 4. Mass concentration of total flavonoids in saturated solutions at different temperatures

Plotting Cs against T (Fig. 4), the relationship between saturation concentration and temperature can be expressed as: Cs = 0.3247T-113.13, R= 0.9944.

Substituting h_0 and Cs into Equation (5), the extraction kinetic equation of dandelion flavonoids can be obtained as:

Ct=t/{1/[exp (127.92-45650 /T)]+t/(0.3247T- 113.13)}

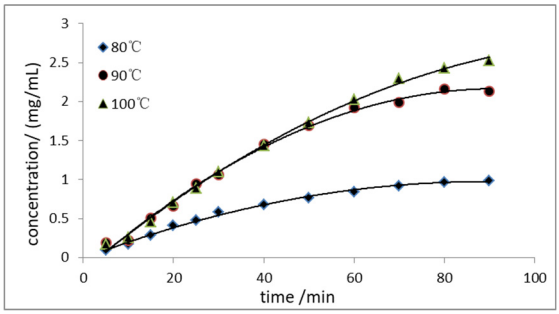


Fig 5. Extraction prediction model for extraction of dandelion flavonoid at arbitrary time and temperature

This equation provides the relationship between extraction time t, extraction temperature T, and extraction concentration Ct. As shown in Fig. 5, with increasing temperature and time, the yield increases. Correctly controlling process conditions during PLE can result in higher flavonoid yields. This result also indicates that high-temperature and high-pressure aqueous alcohol is undoubtedly a suitable solvent, providing efficient mass transfer and high solubility for dandelion flavonoids.

4. Conclusion

In the pressurized extraction process of dandelion flavonoids, the established kinetic equation expresses the relationship between mass concentration, time, and temperature as: $Ct = t /\{1/[\exp (127.92 – 45650 / T)]+t /(0.3247T - 113.13)\}$. This equation is consistent with the characteristics of the second-order kinetic model, indicating that the extraction process proceeds in two steps: first, the solvent penetrates into the matrix and rapidly dissolves the flavonoid components from the cells, and then the flavonoids slowly diffuse from the medicinal materials into the extraction solution. Pressurized extraction technology effectively accelerates the slow process by using increased pressure and temperature, thereby increasing the mass transfer rate. Therefore, based on the equation described in this study, it is possible to predict the concentration of the extract within a certain range, which helps in adjusting the extraction process parameters and provides theoretical guidance for industrial production.

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References

[1] Du JH, Xu MY, Wang Y, Lei Z, Yu Z and Li MY. Evaluation of Taraxacum mongolicum flavonoids in diets for Channa argus based on growth performance, immune responses, apoptosis and antioxidant defense system under lipopolysaccharide stress. Fish Shellfish Immunol. 2022 Dec; 131:1224-1233.

[2] Kang L, Miao MS, Song YG, Fang XY, Zhang J, Zhang YN and Miao JX. Total flavonoids of Taraxacum mongolicum inhibit non-small cell lung cancer by regulating immune function. J Ethnopharmacol. 2021 Dec 5; 281:114514.

[3] Chen RJ, Wang QY, La XJ et al. Progress of medicinal research on Dandelion[J]. Modern Journal of Integrative Chinese and Western Medicine,2021,30(05):563-567.

[4] Gallego R, Bueno M and Herrero M. Sub- and supercritical fluid extraction of bioactive compounds from plants, food-by-products, seaweeds and microalgae – An update[J]. Trends in Analytical Chemistry, 2019, 116198-213.

[5] Djéssica T R, Camila S and Silvio C. Pressurized liquid extraction of compounds from Stevia leaf: Evaluation of process variables and extract characterization[J]. The Journal of Supercritical Fluids, 2023, 193:105813.

[6] F. J G, Gerardo Á, G. H T, et al.Hass Avocado (Persea americana Mill.) residues as a new potential source of neuroprotective compounds using pressurized liquid extraction[J]. The Journal of Supercritical Fluids, 2024,204.

[7] Alvarez-Rivera G, Bueno M, Ballesteros-Vivas D., Mendiola J A and Ibañez E, Pressurized liquid extraction. Liq. Phase Extr. Handb. Sep. Sci. (2020), pp. 375-398.

[8] Yabalak E, Aminzai T M, Gizir M A, et al. A Review: Subcritical Water Extraction of Organic Pollutants from Environmental Matrices[J]. Molecules,2024,29(1).

[9] Peng XJ, Liu N, Wang MX, et al. Recent advances of kinetic model in the separation of essential oils by microwave-assisted hydrodistillation[J]. Industrial Crops and Products, 2022, 187, Part A, 115418.

[10] Andrianony F A, Ravelonandro P H and Rajaona R. Modeling and kinetics study of Avocado oil extraction from Madagascar using different mathematical models[J]. South African Journal of Chemical Engineering, 2022, 41: 93-97.

[11] Ipsita D. and Amit A. Kinetics and mechanistic models of solid-liquid extraction of pectin using advance green techniques- a review[J]. Food Hydrocolloids, 2021, 120: 106931.

[12] Mohamad Y E,Retno N D and Mega M. Kinetics modelling of the solid–liquid extraction process of linamarin compounds from cassava leaves assisted by UV-Photobioextractor[J]. Materials Today: Proceedings, 2023,87(P2):230-233.

[13] Parinaz H, Valentine O O, Christine D, et al. Kinetic modelling of the solid–liquid extraction process of polyphenolic compounds from apple pomace: influence of solvent composition and temperature[J]. Bioresources and Bioprocessing,2021,8(1).

[14] Hanatou A H, Henri F, Catherine P and Ho Y S, Comparison of Kinetic Models for the Aqueous Solid–Liquid Extraction of Tilia Sapwood in a Continuous Stirred Tank Reactor, Chemical Engineering Communications, 2007, 194:4,537-552.