

MATHEMATICAL MODELING OF TOTAL FLAVONOID COMPOUNDS EXTRACTION FROM DILL (*Anethum graveolens* L.) LEAVES

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Abstract: In this paper, extraction of the total flavonoid compounds from dill (*Anethum graveolens* L) leaves using an aqueous ethanol solution (50% by volume) was studied at different temperatures (30, 40, and 50 °C). A physical unsteady-state diffusion model and empirical Ponomarev model were developed to model the extraction process's kinetics. The enthalpy and entropy changes were positive, while the Gibbs free energy was negative and decreased when temperature increased during the thermodynamic analysis. Therefore, extraction of total flavonoids from dill leaves was endothermic, spontaneous, and irreversible.

Keywords: extraction, flavonoids, dill, kinetic, thermodynamic

Introduction

Anethum graveolens L., commonly known as dill, is native to Mediterranean countries. Southeastern Europe is a rich source of phenolic compounds (Kuling et al., 2019). Dill is an essential aromatic herb used to flavor various foods, such as salads, sauces, soups, seafood, and especially pickles. Additionally, dill leaves are also used as dried. In traditional herbal medicine, the dill leaves are also used as a diuretic and solve gastrointestinal problems, such as flatulence, indigestion, stomachache, and colic (Isbilir et al., 2011).

Solid-liquid extraction has been widely used to isolate bioactive compounds from different parts of plants. The extraction efficiency is affected by several factors: the type of solvent and its concentration, the solvent-solid ratio, pH, time of extraction, temperature, and particle size of the solid matrix (Kitanović et al., 2008). Mathematical modeling of the extraction can help in explaining the process, but also in the creation of the optimized extraction process.

This paper aimed to search for the best suited kinetic model to explain the extraction of total flavonoid compounds from dill leaves. Besides, the fitting of each model to the experimental kinetic data was checked using the root mean square error and the correlation coefficient. Thermodynamic parameters (enthalpy, entropy, and Gibbs free energy changes) were also calculated.

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Materials and methods

Materials

In summer, dill samples were harvested by hand (Niš, Serbia). The herb materials were dried immediately after harvesting in a shady and well-aired place for 15 days. The dry plant materials were then packed in paper bags and kept in a dark and cool place. Before being used, the plant material was comminuted by a hammer mill and sieved through a 6 mm screen.

Initial Contents of Flavonoid Compounds in Plant Materials (q_0)

The plant material (2.5 g) was weighed into a 250 mL Erlenmeyer flask with a ground stopper and covered with 100 ml of the extraction agent. The extraction was carried out by the maceration method for 120 minutes. The extracts were separated from the residues by filtering through the Whatman No. 1 filter paper. The sediments were extracted twice with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45°C, using a rotary evaporator (BUCHI Rotavapor R-200). The dried crude concentrated extracts were dissolved using extraction solvent until used for analyses. The experiment was performed in triplicate.

The Kinetics of Extraction

The dill (2.5 g) and the extracting solvent (50 mL) were placed in a series of Erlenmeyer flasks (250 mL). The flasks were macerated in 5, 10, 15, 20, 30, 40, 60, and 80 minutes. The temperature was controlled and maintained at $30 \pm 0.1^\circ\text{C}$. After each time interval, a flask was taken out of the thermostatic bath, and the liquid extract was separated from the plant residues by vacuum filtration. The procedure was repeated at $40 \pm 0.1^\circ\text{C}$ and $50 \pm 0.1^\circ\text{C}$.

Total flavonoids

The total flavonoids (TF) assay was done as previously described by Zhishen et al. (1999). A volume of 1 ml of diluted extract or standard solution of catechin (50-500 mg/L) was placed in a 10 ml volumetric flask, then 4 ml of dd H₂O, and after 5 min 0.3 ml of NaNO₂ (5%) and 1.5 ml of AlCl₃ (2%) were added. The mixture was shaken, and 5 min later, 2 ml of 1 M solution of NaOH were added, again well shaken. The absorbance was measured at 510 nm against the blank. The results were calculated according to the calibration curve for catechin ($R_2 = 0.999$). The content of TF was expressed as mg of catechin equivalent (CE) per gram of FM of dried weight (mg CE/g d.w.). All samples were analyzed in triplicate.

Results and discussion

The extraction kinetics was investigated using the physical unsteady-state diffusion and empirical Ponomarev models. The kinetic parameters of the

extraction are essential for evaluating the flavonoid compounds' extraction potential from dill leaves.

The unsteady-state diffusion model is expressed in Eq. (1) and can be rearranged as a linear Eq. (2)

$$q/q_0 = (1-b) e^{-kt} \tag{1}$$

$$\ln(q/q_0) = \ln(1-b) - kt \tag{2}$$

where q is the content of flavonoid compounds in the liquid extract during the extraction (mg CE/g d.w.), q_0 is the content of flavonoid compounds initially present in the dill leaves (mg CE/g d.w.), k is the slow extraction coefficient of the unsteady-state diffusion model (1/min), and b is the washing coefficient of the unsteady-state diffusion model.

Ponomarev's equation, as being very simple, is frequently used to model the slow extraction period:

$$(q_0 - q)/q_0 = b' + k't \tag{3}$$

where k' is the slow extraction coefficient of the Ponomarev model, and b' is the washing coefficient of Ponomarev's model.

Fig. 1. shows how the flavonoids content changes during the extraction of flavonoids from pumpkin seeds at different temperatures. The flavonoids content increased with the extraction time for all constant extraction temperatures. Two extraction periods can easily be observed: washing, characterized by a rapid increase in the concentration of flavonoids at the beginning of the process, and slow extraction (approximately after the first 10 min), characterized by a slow increase in the concentration.

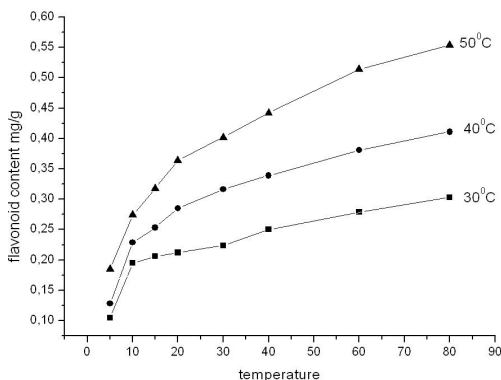


Figure 1: Variation of the flavonoid compounds content in liquid extracts during the extraction process at (■) 30±0.1°C; (●) 40±0.1°C and (▲) 50±0.1°C.

The criteria used to evaluate how well models represent the experimental data and to select the best one was the magnitudes of the linear correlation coefficient and root mean square error (*RMSE*). The higher is the value of linear correlation coefficient and the lower is the value of *RMSE*, the better will be the goodness of fit.

Firstly, the experimental data were put into the linear Eq. (2) for the unsteady-state diffusion model and Eq. (3) for the Ponomarev's model. The parameters obtained as results of a linear regression fit are available in Table 2. The parameters, *b* and *b'* increased with the rise of the temperature. Also, *k* and *k'* showed the same behavior. A similar behavior was observed in previous studies [10,21]. Therefore, kinetic parameters depend on the kinetic model.

Table 1. The statistical values of two models for extraction of flavonoid compounds from dill leaves

Model	Parameters	30°C	40°C	50°C
Unsteady-state diffusion model	b	0.249	0.308	0.369
	k (1/min)	2.15×10^{-3}	3.46×10^{-3}	5.31×10^{-3}
	RMSE (%)	0.35	1.34	2.03
	R ² (%)	99.12	95.20	95.30
Ponomarev model	b'	0.409	0.505	0.590
	k' (1/min)	2.76×10^{-3}	3.43×10^{-3}	4.07×10^{-3}
	RMSE (%)	0.52	1.84	2.89
	R ² (%)	98.11	91.43	91.18

It was evident that individual values of the RMSE were less $\pm 10\%$ for each of the two models considered. Also, from Table 1, it was apparent that the values of the R² were less than 90%. The coefficient of determination, R², is the proportion of variation in the response attributed to the model rather than random error and suggested that R² should not be < 80% for an excellent fitted model.

Based on these results unsteady-state diffusion model with the higher value of the linear correlation coefficient and low RMSE values was proclaimed as the best kinetic model for flavonoid compounds extraction from dill leaves.

Activation energy is an essential parameter for a better understanding of the extraction process. The *E_a* values of flavonoid compounds extraction from dill leaves were obtained from the slope of the plot (*lnk* vs. *1/T*) in the unsteady-state diffusion model. The activation energy needed for the extraction process was 1.875 kJ/mol. Our values were similar to the literature values reported for other bioactive compounds. For the extraction of the polyphenols from grape seeds with 50% aqueous ethanol, the value of the activation energy was 1.10 – 7.70 kJ/mol (Bucić-Kojić et al., 2007).

Gibbs's free energy change for the total flavonoid compounds extraction from dill leaves was negative, -2.683, -2.931, and -3.180 kJ/mol for 30, 40, and 50 °C, respectively. The spontaneous extraction of the flavonoid compounds was favored

with increasing the extraction temperature. A similar tendency was also reported for flavonoids extraction from *Phyllanthus Emblica* (-2.65 to 0.15 kJ/mol) (Krishnan et al., 2017).

The enthalpy change value for the extraction process is 4.852 kJ/mol. The positive enthalpy change indicated that the extraction process was endothermic and, as such, required an external energy source during the extraction. The positive values of entropy change (24.867 K/mol) for the extraction process of flavonoid compounds from dill leaves using the 50% ethanol solution were an indication that the process was irreversible, thus in line with the findings of our previous study (Mitić et al., 2021).

Conclusion

Application of two models (unsteady-state diffusion model and Ponomarev model) showed the unsteady-state diffusion model as suitable because a large value for R^2 ($R^2 > 95.20\%$) and small values for RMSE (RMSE < 2.03%). The total flavonoids extraction from dill leaves was shown by a thermodynamic analysis to be endothermic, spontaneous, and irreversible.

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MATEMATIČKO MODELOVANJE EKSTRAKCIJE TOTALNIH FLAVONOIDA IZ LISTOVA MIROĐIJE (*Anethum graveolens* L.)

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Izvod

U ovom radu proučavana je ekstrakcija ukupnih flavonoida iz listova mirođije (*Anethum graveolens* L) korišćenjem vodenog rastvora etanola (50% zapremine) na različitim temperaturama (30, 40 i 50 °C). Za modelovanje kinetike procesa ekstrakcije razvijeni su modeli difuzije u nestacionarnom stanju i empirijski model Ponomarjeva. Promene entalpije i entropije su bile pozitivne, dok je vrednost Gibsove slobodne energije bila negativna i smanjivala se kako se temperatura povećavala tokom termodinamičke analize. Stoga je ekstrakcija ukupnih flavonoidnih jedinjenja iz listova mirođije bila endotermna, spontana i nepovratna.

Gljučne reči: ekstrakcija, flavonoidi, mirođija, kinetika, termodinamika

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