Research Article

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Kinetic models of the extraction of vanillic acid from pumpkin seeds

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Abstract: Vanillic acid is used in the food industry and perfumery, and the optimization of its extraction process from the natural source is important for saving time and money. The presence of vanillic acid in pumpkin seeds was proven using HPLC analysis. Computational optimization of the extraction shows that for the concentration of ethanol 40% and solmodul: V/m=20, the optimum condition for the extraction of vanillic acid from pumpkin seeds was 100 min and 45°C. The estimation of fitting for each kinetic model to the experimental kinetic data was performed using the root mean square, standard deviation, and the correlation coefficient. Ponomarev model was shown as the most suitable with the highest accuracy among the six considered kinetic models. The enthalpy and entropy changes were positive, while the Gibbs free energy was negative and decreased when temperature increased during the thermodynamic analysis. Therefore, the extraction of vanillic acid from pumpkin seeds was endothermic, spontaneous, and irreversible.

Keywords: Extraction yield; Vanillic acid; Pumpkin seed; Ponomarev model.

1 Introduction

Pumpkin (Cucurbita pepo L.) is one of the vegetables used in healthy diets as well as in traditional medicine in many countries. Pumpkin seeds (known as pepitas) show high nutritional and medicinal values, so it is not surprising that they are often recommended against digestive problems in the folk medicine [1]. Cold-pressed pumpkin seeds oil is a rich source of phytosterols tocopherols, squalene [2,3], and minerals [4].

Pumpkin seeds are a rich source of vanillic acid. Vanillic acid (4-hydroxy-3-methoxy benzoic acid) is a secondary metabolism product in plants. It is widely used as a food additive, preservative, and in perfumery [5]. Vanillic acid exerts strong antioxidant activity, but also hypotensive, cardioprotective, hepatoprotective, and antiapoptotic activities, and even gene regulation roles [6-9]. Solid-liquid extraction has been widely used for the isolation of bioactive compounds from different parts of plants. The efficiency of the extraction 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 [10-13]. Mathematical modeling of the extraction can help in explaining the process, but also in the creation of the optimized extraction process. Kinetic models (either physical or empirical), based on the physical phenomena, might be very complicated. The most often used models are based on film theory and the concept of unsteady-state diffusion through particles: the hyperbolic equation (Peleg’s model), the second-order model, and Elovich’s equation. Ponomarev’s equation, was shown as adequate for the explanation of the slow extraction period [10].

The aim of this paper was searching for the best suited kinetic model for the explanation of the extraction of vanillic acid from pumpkin seeds. Besides, the fitting of each model to the experimental kinetic data was checked using the root mean square, standard deviation, and the correlation coefficient. Thermodynamic parameters (enthalpy, entropy, and Gibbs free energy changes) were found. Finally, the optimization of the extraction process of vanillic acid using computer software JMP (SAS Institute Inc., Cary, USA) was performed.
2 Experimental

2.1 Plant Materials

Pumpkin seed samples were obtained in Serbia from local groceries. Peeled seeds of pumpkin were dried at room temperature and grounded immediately prior to the extraction (average plant particle size was 0.60 mm).

2.2 HPLC-DAD analysis of vanillic acid

Quantification of vanillic acid was determined using reversed phase HPLC method. An HPLC Agilent-1200 series (Agilent Technologies, Santa Clara, California, USA) was used with UV-Vis DAD (Agilent Technologies, Santa Clara, California, USA) for multi-wavelength detection. The column was thermostated at 30°C. After injecting 5 μL of the sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6x150 mm column (Agilent Technologies, Santa Clara, California, USA). Two solvents were used for the gradient elution: A-(H₂O+2% HCOOH) and B-(80% Acetonitrile+5% HCOOH+H₂O). The used elution program was earlier developed by our research group with the flow rate of the mobile phase 0.8 mL/min [14].

The vanillic acid in extracts was identified by comparing retention time and spectra with those of pure component and literature data [1]. Stock standard solution (1 mg/mL) of the vanillic acid (Sigma, St. Louis, MO, USA) was used as a reference standard to monitor the performance of HPLC on a regular basis. Concentrations of vanillic acid in extracts were calculated from equation obtained. All analyses were performed in triplicates.

2.3 The initial content of vanillic acid in pumpkin seeds (q₀)

Pumpkin seeds (2 g) were weighed into a 250 mL Erlenmeyer flask with a ground stopper and covered with 100 mL of the extraction agent (aqueous solution of the ethanol with the concentration 40% (v/v)). The maceration was carried out for 120 min. The extract was separated from the residuals by filtering through the Whatman No. 1 filter paper (Whatman, Maidstone; United Kingdom). The residuals were extracted twice with the same fresh solvent and the extracts combined. The second and the third macerations were carried out each for 30 min. Afterward, they were concentrated under reduced pressure at 45°C using a rotary evaporator (BUCHI Rotavapor R-200, BÜCHI Labortechnik AG, Switzerland). The dried crude concentrated extracts were dissolved using an extraction solvent before the analysis. The experiment was performed in triplicate. Vanillic acid was determined by the HPLC-DAD method quantitatively. The value of the initial content of vanillic acid in pumpkin seeds was 26.905 mg/g.

2.4 The concentration of vanillic acid in pumpkin seeds in the saturated liquid extract (cₘₐₓ)

Pumpkin seeds (2 g) were weighed into a 250 mL Erlenmeyer flask with a ground stopper and covered with 100 mL of the extraction agent (aqueous solution of the ethanol with the concentration 40% (v/v)). The maceration was carried out for 120 min. The liquid extract was separated as before and used for the extraction of vanillic acid from a further portion (2 g) of the fresh pumpkin seeds. The extraction was repeated with the liquid extract and a further quantity of fresh pumpkin seeds. The extraction solvent was evaporated under rotary evaporator at 45°C. The dried crude extracts were dissolved using an extraction solvent (aqueous solution of the ethanol with the concentration 40% (v/v)) and then used for the analyses. The dried extract was prepared in triplicates and the results averaged. The value of the vanillic acid was determined by the HPLC-DAD method, and in saturated liquid extracts was 1.256 g/L, 1.265 g/L and 1.272 g/L at 25°C, 35°C and 45°C, respectively.

2.5 The kinetics of extraction

The pumpkin seeds (2 g) and the extraction solvent (aqueous solution of the ethanol with the concentration 40% (v/v)) (40 mL) were placed in a series of Erlenmeyer flasks (250 mL) and left for 10, 20, 30, 40, 60, 80 and 100 min. The temperature was controlled and maintained at 25±0.1°C. After each time interval, the liquid extract was separated from pumpkin seeds by vacuum filtration. The content of vanillic acid in the liquid extracts was determined by the HPLC-DAD methods. Each liquid extracts were prepared thrice and the results averaged. The procedure was repeated at 35±0.1°C and 45±0.1°C.
2.6 Kinetic model

The extraction kinetics was investigated using the physical models (unsteady-state diffusion model, film theory model) and empirical models (hyperbolic model, second-order model, Elovich model, and Ponomarev model). The kinetic parameters of the extraction are important for evaluating the vanillic acid extraction potential from pumpkin seeds.

The unsteady-state diffusion model is expressed in Eq. (1) and can be rearranged as a linear Eq. (2) [10,15]:

\[
\frac{q}{q_0} = (1 - b)e^{-kt}
\]

\[
\ln\left(\frac{q}{q_0}\right) = \ln(1 - b) - kt
\]

where \( q \) is the content of vanillic acid in the liquid extract during the extraction (mg/g), \( q_0 \) is the content of vanillic acid initially present in the pumpkin seeds (mg/g), \( k \) 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.

The film theory model is expressed in Eq. (3) and can be rearranged as a linear Eq. (4) [16]:

\[
tk'_{ebc} = \frac{(1 - b')}{(1 - b') - k't}
\]

\[
\ln\left(1 - \frac{c}{c_s}\right) = \ln(1 - b') - k't
\]

where \( c \) is the concentration of vanillic acid in the liquid extract during extraction (mg/ml), \( c_s \) is the maximum concentration of vanillic acid in the solvent at temperature (K), \( c_{ebc} \) is the slow extraction coefficient of the film theory model (1/min), and \( b' \) is the washing coefficient of the film theory model.

Ponomarev’s equation, as being very simple, is frequently used to model the slow extraction period [16]:

\[
h = \left. \frac{c}{t} \right|_{t \to 0} = k''_2 c_s^2
\]

where \( h \) is the initial extraction rate (mg/mL min). Elovich’s equation is a logarithmic relation [10]:

\[
\dot{q} = E_0 + E_1 \ln t
\]

A plot of extraction yield \( \dot{q} \) verse \( \ln t \) gives \( E_0 \) as the intercept and \( E_1 \) as the slope.

2.7 Activation energy and thermodynamic parameters

The Arrhenius equation was used for the calculation of the activation energy, which was used to describe the relationship between the \( k \) slow extraction coefficient of the unsteady diffusion model, or \( k'' \) slow extraction coefficient of the Ponomarev model, or \( k_2 \) second-order extraction rate constant and the temperature. It is expressed in Eq. (12), and can be rearranged as a linear Eq. (13)[18]:

\[
q = \frac{K_1 t}{1 + K_2 t}
\]

\[
t = \frac{1}{K_1} + \frac{K_2 t}{K_1}
\]
where \( E_a \) is the activation energy (kJ/mol), \( R \) is the gas constant, and \( T \) is the absolute temperature (K).

Thermodynamic parameters

\[
\ln K_e = -\frac{\Delta H^0}{R} \frac{1}{T} + \frac{\Delta S^0}{R} \tag{14}
\]

\[
K_e = \frac{m_L}{m_S} \tag{15}
\]

where \( K_e \) is equilibrium constant, \( m_L \) is the amount of vanillic acid in liquid at equilibrium temperature (T), \( m_S \) is the amount of vanillic acid in solid at T, \( \Delta H^0 \) (kJ/mol) is enthalpy change, \( \Delta S^0 \) (J/mol K) is entropy change and \( \Delta G^0 \) (kJ/mol) is the standard Gibbs free energy change (kJ/mol).

\( \Delta H^0 \) and \( \Delta S^0 \) can be determined using the slope and y-intercept of the plot of \( \ln K_e \) versus \( 1/T \) based on Eq. 14 [13].

### 2.8 Statistical analysis

The best fit was checked by root mean square [10], standard deviation [19], and the coefficient of determination [20]. The root mean square (RMS) was calculated using equation (16), while the standard deviation (SD) was calculated using equation (17):

\[
RMS = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left( \frac{q_{exp} - q_{cal}}{q_{exp}} \right)^2} \tag{16}
\]

\[
SD = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} \left( \frac{q_{exp} - q_{cal}}{q_{exp}} - AARE \right)^2} \tag{17}
\]

Absolute average relative error (AARE) was calculated using equation (18),

\[
AARE = \frac{1}{N} \sum_{i=1}^{N} \left| \frac{q_{exp} - q_{cal}}{q_{exp}} \right| \tag{18}
\]

If the value of \( R^2 \) is higher and the values of the RMS and SD are lower, the better will be the fit [10].

### 2.9 Response surface methodology and optimum conditions for the extraction

Response surface design and the finding of the optimum conditions for the optimization of the extraction process were achieved using the software JMP 14.0.1 (SAS Institute Inc., Cary, USA). Two factors were selected: time (20 min, 60 min, and 100 min) and temperature (25°C, 35°C, and 45°C), and one response-change of the quantity of vanillic acid in pumpkin seeds, and the Central Composite Design with 2 central points (Table 1). The script for the model was run, and the results were displayed with all statistical data. The optimum conditions regarding time and the temperature were found using the option of Prediction profiler and the selection of Maximize Desirability.

### 3 Results and Discussion

#### 3.1 Vanillic acid variation with extraction time

Fig. 1. shows how the vanillic acid concentration changes during the extraction of vanillic acid from pumpkin seeds at different temperatures.

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**Table 1**: Response surface design for the optimization of the extraction process.

| Pattern | Time (min) | Temperature (°C) | Vanillic acid (mg/g) |
|---------|------------|------------------|----------------------|
| 0a      | 60         | 25               | 9.525                |
| 00      | 60         | 35               | 11.599               |
| A0      | 100        | 35               | 15.008               |
| 0A      | 60         | 45               | 13.32                |
| ++      | 100        | 45               | 17.729               |
| -+      | 20         | 45               | 7.898                |
| a0      | 20         | 35               | 7.05                 |
| --      | 20         | 25               | 5.941                |
| +-      | 100        | 25               | 12.542               |
| 00      | 60         | 35               | 11.599               |
For all constant extraction temperatures, the vanillic acid concentration increased with the extraction time. Two periods of extraction can easily be observed: washing, characterized by a rapid increase in the concentration of vanillic acid at the beginning of the process, and slow extraction (approximately after the first 20 min), characterized by a slow increase in the concentration. Also, the content of vanillic acid increased with the rise of the extraction temperature.

### 3.2 Effects of process variables

The computational modeling of the response surface design and subsequent optimization gives more easily optimized conditions for the extraction of vanillic acid from pumpkin seeds.

Contour profiler of the extraction process for the investigated pumpkin seeds is given in Figure 2.

**Figure 1:** Variation of the vanillic acid concentration in liquid extracts during the extraction process at (•) 25±0.1°C; (●) 35±0.1°C and (▲) 45±0.1°C.

**Figure 2:** Contour profiler of the extraction process of vanillic acid depending on temperature and time with the concentration of ethanol 40% and solmodul: V/m=20.
The optimum condition for the extraction of vanillic acid from pumpkin seeds was 100 min and 45°C.

### 3.3 Kinetics of vanillic acid extraction

Physical and empirical models were applied to analyze the experimental data. The suitability of each model was examined using RMS, SD, and $R^2$, while the extraction was quantitatively estimated using a kinetic analysis.

Firstly, the experimental data were put into the linear Eq. (2) for the unsteady-state diffusion model, linear Eq. (4) for the film theory model and Eq. (5) for the Ponomarev’s model. The parameters obtained as results of a linear regression fit are available in Table 2. The parameters, $k''$, $b''$, and $c''$, increased with the rise of the temperature. Also, $b$, $k$, and $k''$ showed the same behavior. A similar behavior was observed in previous studies [10,21]. Therefore, kinetic parameters depend on the kinetic model. The film theory model gives the smallest values of washing coefficient and the highest values of the slow diffusion coefficient.

The experimental data were also introduced into the linear Eq. (7) of the hyperbolic model, linear Eq. (9) of the second-order model and Eq. (11) for Elovich’s model. The parameters are presented in Table 2. Hyperbolic and second-order models, their kinetic parameters $K_r$, $K_s$, $h$, $k_r$, and $c_r$ respectively, also increased with the rise of the temperature. However, the value of $K/K_s$ decreased when increasing the temperature.

The kinetic parameters $E_o$ and $E_i$ for Elovich’s model followed the different trend with the rise of the temperature. While $E_o$ decreased with the increase of the temperature, $E_i$ increased with the increase of the temperature.

The root mean square, standard deviation, and the linear coefficient of determination, were used to evaluate suitability of the models. Table 2 summarizes the percentage values of the RMS, SD and the linear correlation coefficient for each model. It was evident that individual values of the RMS were less ± 10% for each of the six models considered. Also, from Table 2, it was also evident that the individual values of the SD was less ± 10%.

Based on these results Ponomarev model with the highest value of the linear correlation coefficient and low RMS and SD values was proclaimed as the best kinetic model for vanillic acid extraction from pumpkin seeds.

### 3.4 Thermodynamic parameters

Activation energy is an important parameter for a better understanding of the extraction process. The $E_a$ values of vanillic acid extraction from pumpkin seeds were obtained from the slope of the plot $(lnk$ ($k''$ or $k$) vs $1/T)$ in the unsteady-state diffusion model. Ponomarev model and hyperbolic model for the temperature range under investigation were found to give for the activation energy 3.21 kJ/mol, 3.69 kJ/mol, and 4.08 kJ/mol, respectively. There were no previously published results on the activation energy of vanillic acid from pumpkin seeds. However, 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 [22]. For polyphenols from grape extracted with 60% methanol acidified with HCl the value was 23 kJ/mol [23]. The $E_a$ of the extraction of individual flavonols from green tea was in the range of 30 to 50 kJ/mol [24].

Table 3 shows the values of equilibrium constants and thermodynamic parameters for the vanillic acid extraction process of pumpkin seeds. Firstly, values of $\Delta H^\circ$, and $\Delta S^\circ$, were estimated from the slope and intercept ($lnK$ vs $1/T$), respectively, and then the Gibbs free energy change was calculated from the enthalpy change and entropy change using Eq. (14).

The equilibrium constant ($K$) increased when increasing the temperature, indicating that the equilibrium concentration of vanillic acid was also increased.

The values of enthalpy change and the entropy change for the extraction of vanillic acid from pumpkin seeds using aqueous ethanol were positive in the ranges of the extraction temperature. Thus, this extraction process is endothermic and irreversible. Similar results were obtained for the extraction process for other compounds with a different solvent. For the extraction of flavonoids from *Phyllanthus emblica* with water, the value for enthalpy change was 9.53 kJ/mol and entropy change was 30.93 J/mol K [25]; for total phenolics extraction from hop with aqueous ethanol the values were 7.32 kJ/mol for enthalpy change and 60.74 J/mol K for entropy change [21]; for extraction of Jatropha oil using acidic hexane, the reported $\Delta H^\circ$ and $\Delta S^\circ$ were 0.159 kJ/mol and 15.27 J/mol K [26], respectively, and finally, the values determined for melon and rubber seed oil and olive cake oil extraction were in the range 4–13.5 kJ/mol for enthalpy change [12,27], and sunflower seeds and hempseeds oil extraction in the range 33.09-44.19 J/mol K for entropy change [28,29].

The change of Gibbs free energy for the vanillic acid extraction from pumpkin seeds was negative. The
Table 2: The statistical values of various kinetic models for extraction of vanillic acid from pumpkin seeds.

| Model (period extraction)                          | Parameters | 25 | Temperature (°C) |
|--------------------------------------------------|------------|----|------------------|
| Unsteady-state diffusion model (30-100 min)      | b          | 0.578 | 0.636 | 0.669 |
|                                                  | $k$ (1/min) | $2.94 \cdot 10^{-3}$ | $3.13 \cdot 10^{-3}$ | $3.56 \cdot 10^{-3}$ |
|                                                  | RMS (%)     | 0.56 | 1.23 | 1.59 |
|                                                  | SD (%)      | 0.63 | 1.38 | 1.77 |
|                                                  | $R^2$ (%)   | 99.92 | 99.54 | 99.46 |
| Film theory model (10-100 min)                   | $b'$        | 0.143 | 0.144 | 0.149 |
|                                                  | $k'$ (1/min) | $3.82 \cdot 10^{-3}$ | $5.02 \cdot 10^{-3}$ | $6.66 \cdot 10^{-3}$ |
|                                                  | RMS (%)     | 9.45 | 8.78 | 4.44 |
|                                                  | SD (%)      | 9.63 | 9.38 | 4.75 |
|                                                  | $R^2$ (%)   | 95.59 | 97.42 | 99.18 |
| Ponomarev model (30-100 min)                     | $b''$       | 0.211 | 0.241 | 0.265 |
|                                                  | $k''$ (1/min) | $2.50 \cdot 10^{-3}$ | $3.15 \cdot 10^{-3}$ | $4.02 \cdot 10^{-3}$ |
|                                                  | RMS (%)     | 1.20 | 1.01 | 0.63 |
|                                                  | SD (%)      | 1.33 | 1.12 | 0.93 |
|                                                  | $R^2$ (%)   | 99.54 | 99.81 | 99.96 |
| Hyperbolic model (10-100 min)                    | $K'_1$ (1/min) | 0.0194 | 0.0246 | 0.0298 |
|                                                  | $K'_2$ (1/min) | 0.0106 | 0.0154 | 0.0207 |
|                                                  | $K'_1/K'_2$ | 1.836 | 1.597 | 1.439 |
|                                                  | RMS (%)     | 4.09 | 5.25 | 7.47 |
|                                                  | SD (%)      | 4.38 | 5.66 | 8.03 |
|                                                  | $R^2$ (%)   | 99.18 | 97.83 | 97.52 |
| Second-order model (10-80 min)                   | $h$ (mg/ml min) | 0.0212 | 0.0270 | 0.0339 |
|                                                  | $k_1$ (mg/ml min) | 0.0645 | 0.0646 | 0.0685 |
|                                                  | $c_s$(mg/ml) | 0.568 | 0.647 | 0.704 |
|                                                  | RMS (%)     | 5.07 | 3.59 | 5.77 |
|                                                  | SD (%)      | 5.02 | 3.93 | 6.30 |
|                                                  | $R^2$ (%)   | 97.84 | 98.42 | 96.58 |
| Elovich model (10-80 min)                         | $E_s$       | 0.865 | 0.842 | 0.815 |
|                                                  | $E_1$       | 0.125 | 0.149 | 0.173 |
|                                                  | RMS (%)     | 2.26 | 3.58 | 5.82 |
|                                                  | SD (%)      | 3.24 | 3.92 | 6.35 |
|                                                  | $R^2$ (%)   | 99.20 | 98.88 | 97.90 |
spontaneity of the vanillic acid extraction was favored with increasing of the extraction temperature. A similar tendency was also reported for flavonoids extraction from *Phyllanthus emblica* (-2.65 to -0.15 kJ/mol) [25], total phenolics from hop (-11.99 to -10.78 kJ/mol) [21], coconut oil (-1.16 to -0.12 kJ/mol) [13], olive cake oil (-6.25 to -4.45 kJ/mol) [12], and hempseeds oil (-5.17 to -2.41 kJ/mol) [28].

### 4 Conclusion

The experimental conditions during the extraction of vanillic acid from pumpkin seeds significantly influence its content. Application of different physical models (unsteady-state diffusion model, film theory model) and empirical models (hyperbolic model, second-order model, Elovich model and Ponomarev model) showed the Ponomarev model as the most suitable (*R*² > 99.54%) and small values for RMS (RMS < 1.20%) and SD (SD < 1.33%). The vanillic acid extraction was shown by a thermodynamic analysis to be endothermic, spontaneous, and irreversible. The performed study enables the researchers to choose the optimal extraction conditions saving money and time dedicated to the research.

**Conflict of interest:** The authors declare no conflicts of interest.

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**Authors’ Contributions:** MM, SJ, PM, JM, JJ: Extraction and kinetic models, manuscript writing; BA: Computational optimization of the extraction, manuscript writing

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### Table 3: Thermodynamic parameters for the extraction of vanillic acid from pumpkin seeds.

| *T* (K) | *K* | Δ*H* (kJ/mol) | Δ*S* (J/mol K) | Δ*G* (kJ/mol) |
|--------|-----|---------------|----------------|---------------|
| 298    | 0.875 | 2.48         | 26.38         | -5.38          |
| 308    | 1.262 |              |               | -5.64          |
| 318    | 1.927 |              |               | -5.91          |
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