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Optimization of Extraction of Phenolic Compounds from Flax Shives by Pressurized Low-Polarity Water

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Pressurized low-polarity water (PLPW) extraction of phenolic compounds from flax shive was investigated using statistically based optimization and the "one-factor-at-a-time" method. Extraction variables examined using central composite design (CCD) included temperature, flow rate, and NaOH concentration of the extracting water. Extraction of phenolic compounds including *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, acetovanillone, and feruric acid was affected by temperature and NaOH concentration; and extraction of all phenolic compounds, except ferulic acid, increased with temperature and NaOH concentration of the extracting water. Flow rate had little effect on concentration of phenolic compounds at equilibrium, but the extraction rate at the early phase was higher for higher flow rates. The mechanism of PLPW extraction of flax shive phenolics was also investigated using a two-site kinetic model and a thermodynamic model. To determine the extraction mechanism, flow rate was varied from 0.3 to 4.0 mL/min while temperature and NaOH concentration were fixed at 180 °C and 0.47 M, respectively. The flow rate tests showed the extraction rates of total phenolic (TP) compounds increased with flow rate and can be described by a thermodynamic model. The results from the thermodynamic model demonstrated that a K_D value of 30 agreed with the experimental data in the flow rate range of 0.3−4.0 mL/min. When the effect of the three independent variables was evaluated simultaneously using CCD, a maximum TP concentration of 5.8 g/kg of dry flax shive (DFS) was predicted from the combination of a high temperature (230.5 °C), a high initial concentration of NaOH (0.63 M), and a low flow rate (0.7 mL/min). Maximum TP concentration of 5.7 g/kg of DFS was obtained from extraction conditions of 180 °C, 0.3 or 0.5 mL/min, and 0.47 M NaOH at equilibrium. A second-order regression model generated by CCD predicted a maximum TP concentration of 5.8 g/kg of DFS under the same extraction conditions, which is well matched with the results from experimental data.

KEYWORDS: Phenolics; *p*-hydroxybenzaldehyde; vanillic acid; vanillin; acetovanillone; ferulic acid; subcritical water; response surface; *Linum usitatissimum*; lignocellulose; lignin

INTRODUCTION

The vast majority of the carbon fixed by photosynthesis is deposited as lignocellulosic material in plants (*1*, *2*). Among the components of lignocellulose, lignin is the second most abundant organic compound in the plant kingdom. Chemically, lignin is a very stable and complex irregular phenolic polymer formed by random polymerization of various precursors, including ferulic acid, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin contains considerable amounts of phenolic acids, which play a major role in the linkage of polysaccharides with lignin by ester and ether bonds (*3*). Hemicellulose, consisting of xylose, arabinose, galactose, and mannose, cross-linked with lignin is considered to be one of the most chemically and biologically resistant biomaterials (*4*−*6*).

Flax, *Linum usitatissimum*, is abundant in North America and is grown mainly for the production of seed used as a source of linseed oil. The stalk, which can be processed into fiber and lignin-rich residue, is referred to as flax shive (*7*). The amount of lignocellulosic materials, including cellulose, hemicellulose, and lignin, in flax shive may vary with region of cultivation, flax age, and analytical methodology (**Table 1**). Even though the flax shive contains about 90% lignocellulosic material including 25% lignin, most of it is used for horse bedding and animal litter (*8*).

In recent years, interest in the use of lignocellulosic materials in flax shive as a source of fermentable sugars and phenolic compounds for the production of bioethanol and fine chemicals has grown due to its abundance and renewability. Phenolic compounds produced by hydrolysis of lignin in flax shive are promising chemicals due to their growing demand in food, fragrance, and polymer industry. However, covalent cross

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Table 1. Chemical Composition of Flax Fiber and Shive

	chemical composition (% total)				
	cellulose	hemicellulose	lignin	ash	refs
fiber	78	6	5	2	*8*
shive	53	13	24	>2	

linkages to polysaccharides by ester bonds and to lignin by ether bonds make hydrolysis difficult and costly (*9*, *10*). The extraction of phenolic compounds using organic solvents, such as ethanol, methanol, ethyl acetate, acetone, or methylene chloride, is widely used (*11*). However, this practice can be costly and environmentally unfavorable. In addition, strict health regulations and increased public concern about organic solvents in food and cosmetic products demand environmentally friendly extraction processes (*12*). The use of lignocellulosic enzymes, including cellulase, xylanase, pectinase, polygalacturonase, and ferulic acid esterase, for the extraction of phenolic compounds from agricultural byproducts has been investigated by many researchers (*10*, *13*−*15*). However, this technique is restricted by low yield, long extraction time, specificity of enzymes, high cost of enzymes, and limited growth conditions (*16*, *17*).

Because chemical and biological extraction methods have several drawbacks, the development of novel processes such as pressurized low-polarity water (PLPW) extraction, which are recognized as being more environmentally acceptable, offers an opportunity for developing new bioproducts and bioprecesses (*18*, *19*). At ambient temperature, water has high polarity (dielectric constant, $\epsilon = 80$), and thus it is not adequate for the extraction of nonpolar compounds. However, the properties of water can be modified by increasing temperature under pressure, a technique known as PLPW extraction, also known as hot compressed liquid, subcritical water extraction, or liquid hot-water extraction (*20*). The dielectric constant, viscosity, and surface tension of water can be lowered by increased temperatures under pressure to keep water in the liquid phase, allowing extraction of low-polarity or nonpolar compounds (*21*, *22*). Several researchers have shown the efficiency of the PLPW extraction for the extraction of compounds such as polyphenols and terpene from a variety of plant tissues (*19*, *23*−*25*). Because PLPW extraction offers the advantages of using water, increased selectivity, cleanliness, fast extraction, and high efficiency, the technique has received much attention for the extraction of phytochemicals from agricultural byproducts.

Phenolic compounds, such as ferulic and vanillic acids and vanillin, have received considerable interest in the past decades due to their feasibility for bioconversion into value-added products (*26*−*29*). The objective of this investigation was to study the application of PLPW extraction of phenolic compounds, namely, *p*-hydroxybenzaldehyde (HBA), vanillic acid (VA), syringic acid (SA), vanillin (VN), acetovanillone (AVN), and ferulic acid (FA), for the production of biochemicals from flax shive and/or the bioconversion of phenolics into fine chemicals such as vanillin and 4-vinylguaiacol. In addition, our goal was to determine the optimal extraction parameters of PLPW extraction, including temperature, NaOH concentration, and flow rate, and to evaluate the mechanism of PLPW extraction of phenolic compounds from flax shive.

MATERIALS AND METHODS

Samples. Flax shive was obtained from Biolin Research Inc. (Saskatoon, Canada). It was ground with a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) using a 0.35 mm blade gap and a 4 mm screen. Bulk ground flax shive was screened using 8 mesh (2 mm opening) and 18 mesh (1 mm opening) screens. The screened flax shive with a particle size between 1 and 2 mm was further separated by air flotation to remove residual fiber. The ground flax shive was sealed and stored in a freezer at −25 °C.

PLPW Extraction. Equipment used for PLPW extraction consisted of an HPLC pump (510 model, Waters, Milford, MA), a 1.0 m preheating coil, extraction cell, a temperature-controlled oven (5700A series, Hewlett-Packard, Palo Alto, CA), a 2.0 m cooling coil, a back pressure regulator with a cartridge of 5.2 MPa (750 psi) (Upchurch Scientific, Oak Harbor, WA), and a collection vessel. All experiments were conducted using the same extraction cell (100 mm long × 9 mm i.d. × 12 mm o.d.), which was manufactured in our mechanical shop. Stainless steel tubing ($^1/_{16}$ in., 1.59 mm o.d. × 0.762 mm i.d.) was used to connect the HPLC pump to the extraction cell, and $^1/_8$ in. (3.175 mm o.d. × 1.580 mm i.d.) tubing was used to connect the extraction cell to the cooling coil. Connections, fittings, extraction cell, and tubing were made of stainless steel and adequate for pressures up to 34 MPa (4900 psi). To maintain identical porosity, equal weights of ground flax shive (0.85 g) and bed depths of 9.3 mm were used in all extractions. To keep the ground flax shive sample inside the extraction cell, glass wool (5 mm thick) was placed at both ends of the extraction cell. The extraction cell was fitted with a 20 μm and a 100 μm frit at the inlet and outlet, respectively, and was mounted in the oven. The extraction procedure was initiated by disconnecting the top fitting of the extraction cell, filling the extraction cell with water containing NaOH at a constant flow rate of 1 mL/min. Once the NaOH solution started to come out of the outlet of the extraction cell, the pump was stopped and the cell was connected to the top fitting. To check for leaks, the system was pressurized to 10.3 MPa (1500 psi) with the outlet valve kept closed at this pressure for 1 min. After completion of the leak test, the outlet valve was opened and the oven was heated to the desired temperature. Extraction was started by pumping the solvent at the desired flow rate and at 5.2 MPa (750 psi). The first 10 mL of NaOH solution, which contained no analyte (dead volume), was discarded, and a total of 50 mL of extract was collected for all experiments. At the end of each extraction, the extraction cell was removed and the system was washed by pumping through 50 mL of 50:50 (v/v) ethanol/tetrahydrofuran solvent mixture and then rinsed with 100 mL of Milli-Q water. Extracts collected from each experiment were stored at −25 °C, and the solid residues were removed from the extraction cell, weighed, dried in a vacuum oven at 60 °C for 24 h, and stored at −25 °C.

Analysis of Phenolics. Analysis of phenolic compounds was conducted on an Agilent 1100 HPLC system with a G1329A autosampler and a G1312A pump, which was controlled by Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). An HPLC method was set up to analyze phenolic compounds, using a Luna C18 5 μm, 150 × 3.0 mm column with a C18 Security Guard cartridge, both from Phenomenex (Torrance, CA). The injector and column temperatures were set at 35 °C, and the injection volume was 20 μL. The mobile phases consisted of methanol (solvent A) and 4.4% (v/v) formic acid (solvent B) with a gradient of $t = 0$ min of 10% A, $t = 30$ min of 25% A, $t = 40$ min of 35% A, $t = 50$ min of 60% A, $t = 60$ min of 100% A, and $t = 70$ min of 10% A. The gradient was optimized for high-quality separation of a mixture of 20 low molecular weight phenolic compounds, which were detected with a diode array detector at 280 nm. Phenolic compounds were identified and quantified by comparison to standards obtained from Extrasynthese (Genay, France) and Sigma Chemicals Co. (St. Louis, MO).

Experimental Design. The effects of three variables (temperature, NaOH concentration, and flow rate) were investigated using a statistical method to maximize extraction yield of six phenolic compounds (*p*-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, acetovanillone, and feruric acid) by Design Expert 6.0 (Stat-Ease Inc., Minneapolis, MN). The central composite design (CCD) required five coded levels for three variables (**Table 2**). The values of each variable (X_i) associated with each coded level were as described (*30*)

$$X_i = \chi_i \Delta X_i + X_{cp} \quad (1)$$

where $i = 1$, 2, or 3 corresponds to each of the three variables; $\chi_i =$

Table 2. Actual and Coded Values of Three Variables in CCD[a]

		coded and actual level				
variable	component	–1.68	–1	0	+1	+1.68
X_1	temperature (°C)	129.5	150	180	210	230.5
X_2	NaOH (M)	0	0.125	0.313	0.5	0.63
X_3	flow rate (mL/min)	0.7	1.0	1.5	2.0	2.3

[a] Experiments conducted with 0.85 g of ground flax shive in extraction cell (100 mm long × 9 mm i.d.) with 5.2 MPa, and 50 mL of extract was collected.

dimensionless coded level for X_i, namely, −1.68, −1, 0, 1, and +1.68; X_i = real value of the independent variable; and X_{cp} = level of the independent variable at the coded value 0. The X_{cp} and ΔX_i values were approximated from preliminary "one-factor-at-a-time" experiments.

The CCD consisted of 17 experimental runs, including 3 replicates of the center points, and was subjected to multiple nonlinear regression using Design Expert 6.0 (**Table 3**). The experimental concentrations of phenolic compounds in the extracts were used to develop a second-order regression model capable of predicting optimum extraction conditions for temperature, NaOH concentration, and flow rate. Design Expert 6.0 provided the analysis of variance (ANOVA) and estimated the coefficient parameters of the regression model. The model predicted optimum conditions for the extraction of *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, acetovanillone, ferulic acids, and the sum of these six phenolic (TP) compounds using coded values of the three variables. Experimental data were fitted to the second-order regression equation

$$Y = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_3 + \beta_{11}\chi_1^2 + \beta_{22}\chi_2^2 + \beta_{33}\chi_3^2 + \beta_{12}\chi_1\chi_2 + \beta_{13}\chi_1\chi_3 + \beta_{23}\chi_2\chi_3 \quad (2)$$

where Y = the predicted response; β_0 = the intercept; β_1, β_2, β_3 = linear coefficients; β_{11}, β_{22}, β_{33} = squared coefficients; β_{12}, β_{13}, β_{23} = interaction coefficients; and χ_i and χ_j = the coded level of variable χ_i and χ_j.

Extraction Models. For the PLPW extraction of essential oil and polycyclic aromatic hydrocarbons, two simple models, thermodynamic and kinetic, have been proposed to explain the extraction mechanism. The two-site kinetic model emphasizes diffusion in the particle, and extraction is limited by diffusion of solutes from the matrix. The thermodynamic model is mainly governed by partitioning of solutes from matrix to water rather than diffusion in the solid matrix. On the basis of the above assumptions, the two models are defined by the following equations (*31*):

$$\text{kinetic model: } \frac{S_T}{S_0} = 1 - [F\,e^{-k_1 t}] - [(1-F)\,e^{-k_2 t}] \quad (3)$$

$$\text{thermodynamic model: } \frac{S_b}{S_0} = \frac{\left(1 - \frac{S_a}{S_0}\right)}{\left[\frac{K_c m}{(V_b - V_a)d} + 1\right]} + \frac{S_a}{S_0} \quad (4)$$

In eqs 3 and 4, S_a = cumulative mass of the analyte extracted after volume V_a (mL), S_b = the cumulative mass of the analyte extracted after volume V_b, S_0 = the initial total mass of analyte in the matrix, S_b/S_0 and S_a/S_0 = the cumulative fraction of the analyte extracted by the fluid of the volume V_b and V_a, S_T = the mass of the analyte removed by the extraction fluid after time t, K_D = the distribution coefficient (concentration in matrix/concentration in fluid), F = fraction of the analyte released, k_1 and k_2 = the first-order rate constant (min^{-1}), d = the density of extraction fluid at given condition (g/mL), and m = the mass of the extracted sample (g).

The two-site kinetic model does not include solvent volume, and the extraction of solutes is independent of flow rate when the extraction profiles are plotted versus time. Conversely, the thermodynamic model contains the factor volume of solvent used, and the extraction rate can be varied by changing the flow rate when the concentration of phenolic compounds is plotted as a function of a extraction time. The mechanisms of thermodynamic elution and diffusion kinetics can simply be compared by changing the flow rate. If the concentration of phenolic compounds in the extract increases with the increase in flow rate at a given extraction time, the extraction mechanism can be explained by thermodynamic model. However, if the increase of flow rate has no significant effect on the extraction of phenolic compounds, the extraction mechanism can be modeled by the two-site kinetic model (*31*). Thus, to investigate the effect of flow rate and evaluate the extraction mechanism, experiments using a "one-factor-at-a-time" method were carried out at different flow rates (0.3, 0.5, 1, 2, and 4 mL/min) at a fixed temperature of 180 °C and at the NaOH concentration of 0.47 M. The extractions were performed sequentially and collected in glass tubes at certain time intervals. Extractions carried out at 0.5 and 4 mL/min were repeated three times, and extracts from five different flow rates were analyzed as described previously.

RESULTS AND DISCUSSION

Values of the independent process variables (X_1, X_2, and X_3) studied and concentrations of the extracted phenolics are shown in **Table 3**. Experimental values of phenolic compounds in the extracts were analyzed by multiple regression to fit the second-order regression equation (eq 2), and seven second-order equations for the concentration of HBA, VA, SA, VN, AVN, FA, and TP were generated as follows:

$$Y_{HBA} = 0.026 + 0.0065\chi_1 + 0.0075\chi_2 - 0.0006\chi_3 + 0.0016\chi_1\chi_2 + 0.0010\chi_1\chi_3 - 0.0015\chi_2\chi_3 + 0.0012\chi_1^2 - 0.0013\chi_2^2 + 0.0019\chi_3^2 \quad (5)$$

$$Y_{VA} = 0.67 + 0.23\chi_1 + 0.089\chi_2 - 0.048\chi_3 - 0.039\chi_1\chi_2 - 0.0009\chi_1\chi_3 - 0.014\chi_2\chi_3 - 0.020\chi_1^2 - 0.037\chi_2^2 + 0.029\chi_3^2 \quad (6)$$

$$Y_{SA} = 0.19 + 0.041\chi_1 + 0.018\chi_2 - 0.018\chi_3 + 0.0140\chi_1\chi_2 + 0.032\chi_1\chi_3 - 0.026\chi_1^2 - 0.024\chi_2^2 \quad (7)$$

$$Y_{VN} = 2.26 + 0.59\chi_1 + 0.53\chi_2 - 0.079\chi_3 - 0.025\chi_1\chi_2 + 0.090\chi_1\chi_3 - 0.12\chi_2\chi_3 - 0.12\chi_1^2 - 0.21\chi_2^2 + 0.081\chi_3^2 \quad (8)$$

$$Y_{AVN} = 0.27 + 0.20\chi_1 + 0.10\chi_2 - 0.0034\chi_3 + 0.056\chi_1\chi_2 - 0.011\chi_1\chi_3 - 0.026\chi_2\chi_3 + 0.033\chi_1^2 - 0.014\chi_2^2 + 0.021\chi_3^2 \quad (9)$$

$$Y_{FA} = 0.12 - 0.015\chi_1 + 0.027\chi_2 - 0.020\chi_1\chi_2 + 0.014\chi_1\chi_3 - 0.020\chi_1^2 - 0.0059\chi_2^2 - 0.012\chi_3^2\chi_1^2 \quad (10)$$

$$Y_{TP} = 3.52 + 1.05\chi_1 + 0.741\chi_2 - 0.174\chi_3 - 0.0065\chi_1\chi_2 + 0.12\chi_1\chi_3 - 0.16\chi_{23} - 0.16\chi_1^2 - 0.26\chi_2^2 + 0.12\chi_3^2 \quad (11)$$

In eqs 5−11, χ_1, χ_2, and χ_3 correspond to the coded values of the three independent variables of temperature, NaOH concentration, and flow rate.

The quality of fit of the second-order regression equations was checked using the coefficient of determination (R^2) and found to be 0.94, 0.91, 0.79, 0.88, 0.98, 0.83, and 0.90 for HBA, VA, SA, VN, AVN, FA, and TP, respectively (**Table 4**). The coefficients for HBA, VA, VN, AVN, FA, and TP indicate good agreement between the observed and predicted responses. The statistical significance of the second-order equations was determined using analysis of variance (ANOVA). The F values imply that the seven equations can adequately predict the

Table 3. Independent Process Variables and Experimental Data for Three-Factor and Five-Level Response Design for PLPW Extraction of Phenolics from Flax Shive[a]

	independent variables			dependent variables						
expt	X_1 (°C)	X_2 (M)	X_3 (mL/min)	HBA (g/kg of DFS)	VA	SA	VN	ACV	FA	TP
1	150.0	0.13	1.0	0.018	0.251	0.201	1.021	0.066	0.055	1.612
2	210.0	0.13	1.0	0.026	0.891	0.163	2.132	0.375	0.053	3.640
3	150.0	0.50	1.0	0.029	0.491	0.104	1.826	0.159	0.143	2.752
4	210.0	0.50	1.0	0.045	0.910	0.209	2.971	0.768	0.029	4.933
5	150.0	0.13	2.0	0.017	0.266	0.043	1.006	0.061	0.065	1.458
6	210.0	0.13	2.0	0.031	0.837	0.218	2.621	0.398	0.091	4.196
7	150.0	0.50	2.0	0.024	0.385	0.074	1.490	0.121	0.111	2.205
8	210.0	0.50	2.0	0.042	0.869	0.219	2.842	0.614	0.081	4.667
9	129.5	0.31	1.5	0.018	0.318	0.045	1.063	0.059	0.104	1.608
10	230.5	0.31	1.5	0.037	0.816	0.150	2.544	0.636	0.037	4.219
11	180.0	0.00	1.5	0.004	0.280	0.031	0.240	0.000	0.048	0.603
12	180.0	0.63	1.5	0.037	0.731	0.183	2.903	0.413	0.204	4.470
13	150.0	0.13	0.7	0.018	0.251	0.201	1.021	0.066	0.055	1.612
14	210.0	0.13	2.3	0.026	0.891	0.163	2.132	0.375	0.053	3.640
15	180.0	0.31	1.5	0.024	0.601	0.189	2.100	0.277	0.124	3.315
16	180.0	0.31	1.5	0.028	0.674	0.178	2.288	0.262	0.129	3.559
17	180.0	0.31	1.5	0.027	0.642	0.177	2.233	0.271	0.116	3.467

[a] X_1, temperature; X_2, NaOH concentration; X_3, flow rate; HBA, *p*-hydroxybenzaldehyde; VA, vanillic acid; SA, syringic acid; VN, vanillin; ACV, acetovanillone; FA, ferulic acid; TP, total phenolic compounds (sum of concentration of HBA, VA, SA, VN, ACT, and FA).

Table 4. ANOVA Table for Second-Order Regression Models

		F value	*p* value[a]	R^2
HBA	model	11.73	0.0019*	0.938
	X_1	40.99	0.0004*	
	X_2	53.70	0.0002*	
	X_3	0.33	0.5810	
VA	model	7.54	0.0072*	0.907
	X_1	52.53	0.0002*	
	X_2	8.30	0.0236*	
	X_3	2.38	0.1667	
SA	model	2.89	0.0881*	0.788
	X_1	11.01	0.0128*	
	X_2	1.93	0.2078	
	X_3	2.16	0.1851	
VN	model	5.88	0.0146*	0.883
	X_1	26.13	0.0014*	
	X_2	20.53	0.0027*	
	X_3	0.48	0.510	
AN	model	31.63	< 0.0001*	0.976
	X_1	200.01	< 0.0001*	
	X_2	57.46	0.0001*	
	X_3	6.01	0.0440*	
FA	model	3.66	0.0507**	0.825
	X_1	5.05	0.0594**	
	X_2	12.17	0.0102*	
	X_3	0.87	0.3818	
TP	model	7.21	0.0081*	0.903
	X_1	38.06	0.0005*	
	X_2	20.66	0.0027*	
	X_3	1.06	0.3365	

[a] *, significance was established at $p < 0.05$; **, significance was established at $p < 0.1$.

experimental results. The probability (*p*) values of HBA, VA, VN, AVN, FA, and TP for temperature and NaOH concentration were <0.1, indicating significant effects of temperature and NaOH concentration on responses. The coefficients of temperature and NaOH concentration were positive except for ferulic acid, implying that higher levels of temperature and NaOH concentration would result in higher recovery of phenolic compounds. Therefore, extraction temperature and NaOH concentration were found to have significant positive effects on the extraction of phenolic compounds from flax shive. The order of significance of process variables affecting phenolic compounds extraction can be ranked as follows: temperature > NaOH concentration > flow rate.

Interactions between Variables. To evaluate the combined effect of two significant variables, namely, temperature and NaOH concentration, three-dimensional response surface curves were plotted using the second-order equations. For each curve, temperature and NaOH concentration were varied from 129.5 to 230.5 °C and from 0.0 to 0.63 M, respectively. The nonsignificant factor, flow rate, was fixed at the central experimental level of 1.5 mL/min. Panels **A** and **B** of **Figure 1** present the effect of temperature and NaOH concentration on *p*-hydroxybenzaldehyde and acetovanillone extraction. Plots show that the concentrations of these two phenolic compounds were affected by temperature and the concentration of NaOH. When the concentration of NaOH increased from 0 to 0.63, a moderate increase in *p*-hydroxybenzaldehyde and acetovanillone was observed at 129.5 °C, whereas a larger increase was found at 230.5 °C. Maximum concentrations of *p*-hydroxybenzaldehyde and acetovanillone of 0.06 and 1.0 g/kg of DFS were achieved at 230.5 °C with a NaOH concentration of 0.63 M. The influence of temperature and NaOH concentration on the extraction of vanillic acid and vanillin at the flow rate of 1.5 mL/min is illustrated in panels **C** and **D**, respectively, of **Figure 1**. The predicted concentrations of phenolic compounds increased with temperature and NaOH concentration, whereas concentrations of vanillic acid and vanillin increased up to 0.47 M NaOH and remained constant or decreased slightly thereafter. Maximum concentrations of vanillic acid and vanillin, 1.0 and 3.0 g/kg of DFS, were predicted with the concentration of 0.47 M NaOH and an extraction temperature of 230.5 °C. **Figure 1E** shows the response surface plot for ferulic acid extraction as a function of temperature and NaOH concentration at a flow rate of 1.5 mL/min. The pattern of PLPW extraction of ferulic acid by PLPW was different from that of the other phenolic compounds with the concentration reaching a maximum value at 150 °C (instead of 230.5 °C) and decreasing considerably thereafter. Thus, the highest amount of ferulic acid, 0.19 g/kg of DFS, was obtained at 150 °C and at a NaOH concentration of 0.63 M.

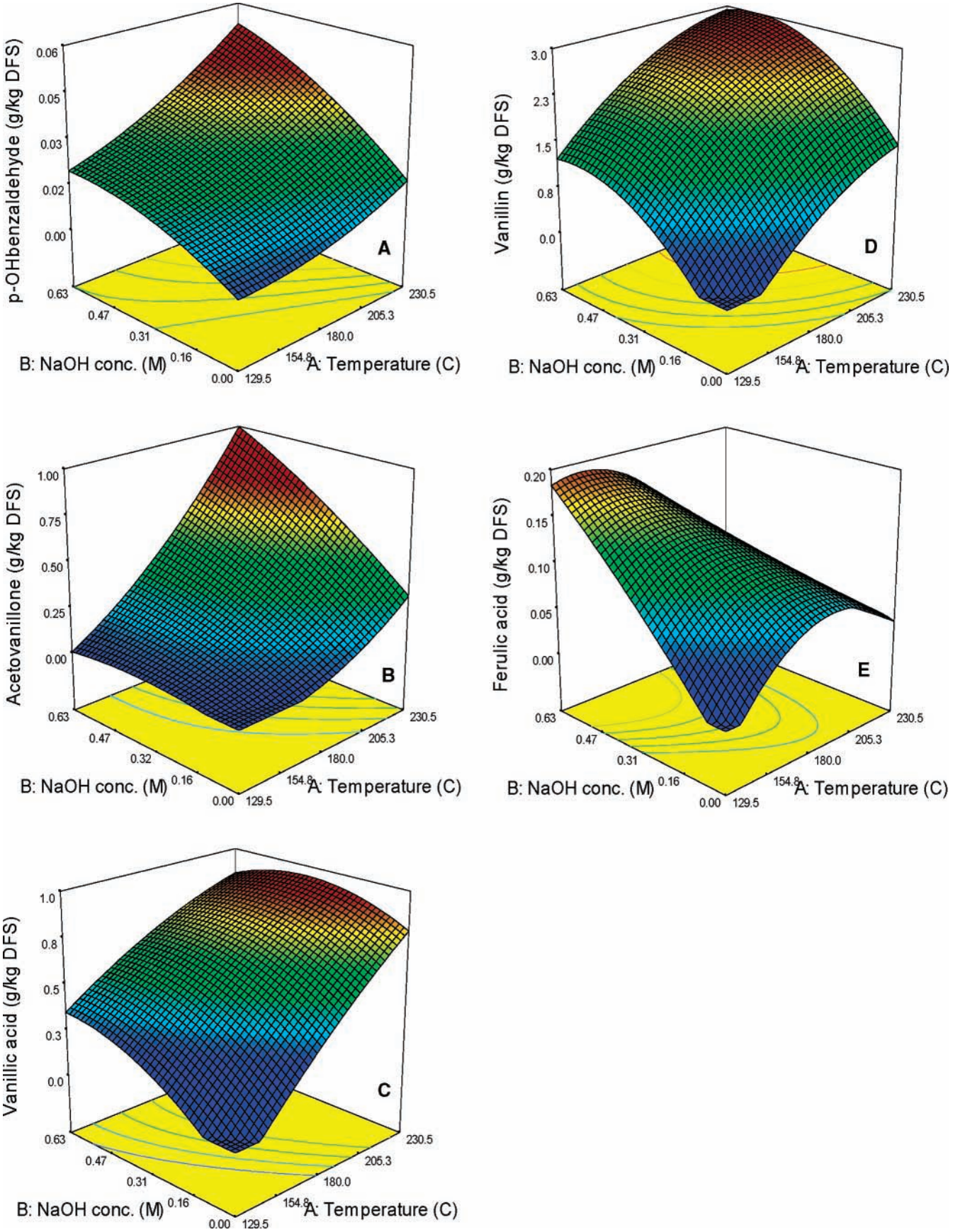

Figure 1. Effect of temperature and NaOH concentration on PLPW extraction of phenolic compounds from 0.85 g of ground flax shive. Flow rate, 1.5 mL/min; pressure, 5.2 MPa; extraction cell, 100 mm long × 9 mm i.d.; volume of extract collected, 50 mL for all experiments.

The effects of temperature and NaOH concentration on TP concentration (sum of HBA, VA, SA, VN, AVN, and FA) at the flow rate of 1.5 mL/min are shown in **Figure 2A**. The extraction trend for recovery of TP was similar to those of

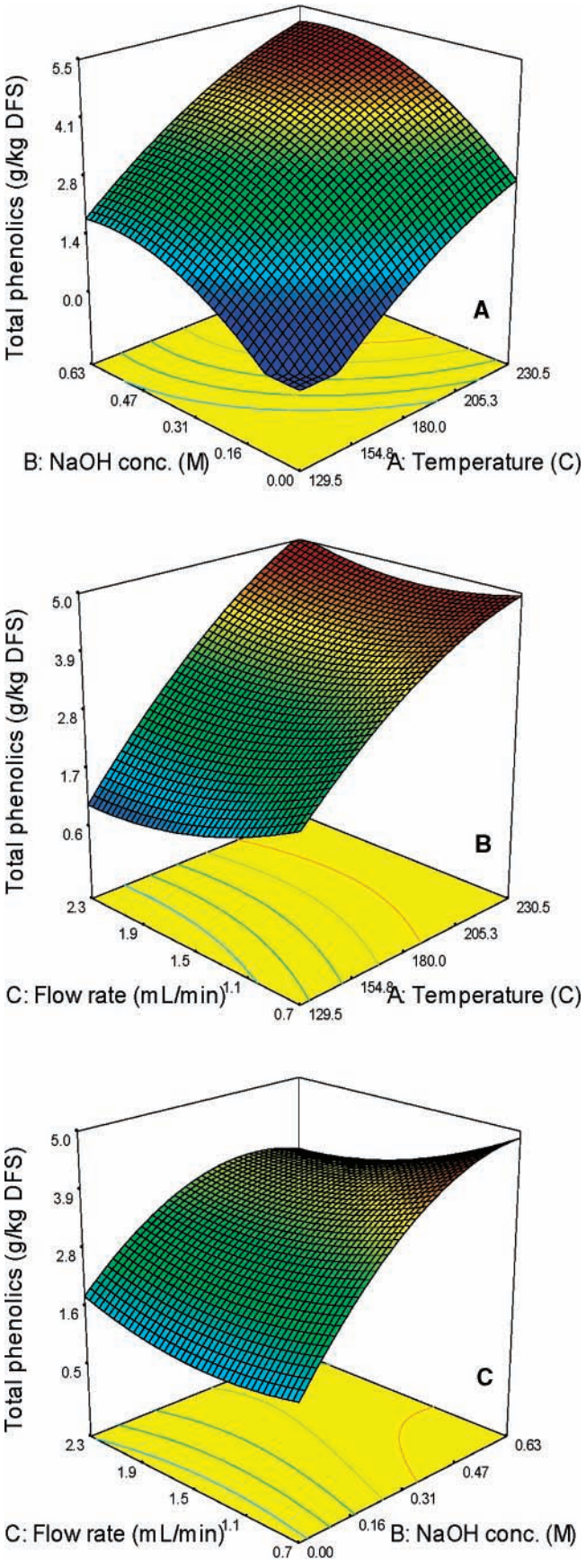

Figure 2. Effect of temperature and NaOH concentration on PLPW extraction of TP (sum of HBA, VA, SA, VN, AVN, and FA) from 0.85 g of ground flax shive. Flow rate, 1.5 mL/min; pressure, 5.2 MPa; extraction cell, 100 mm long × 9 mm i.d.; volume of extract collected, 50 mL for all experiments.

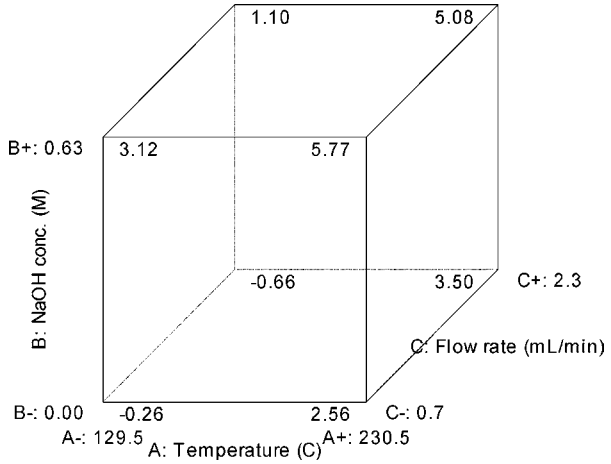

Figure 3. Combined effect of temperature, NaOH concentration, and flow rate on PLPW extraction of TP concentration from 0.85 g of ground flax shive at a pressure of 5.2 MPa in a 100 mm long × 9 mm i.d. extraction cell. The levels of each variable were varied from a coded level of −1.68 to +1.68.

vanillic acid and vanillin, which were the two most abundant components of TP. The concentration of extracted TP increased with temperature and NaOH concentration and reached a maximum value (5.2 g/kg of DFS) at 230.5 °C and 0.5 M NaOH. **Figure 2B**,**C** shows the response surface plots for the extraction of TP as a function of two variables with the other factor being at fixed value. Consistent with the results shown in the previous plots, increase in temperature at a fixed concentration of NaOH led to a considerable increase in TP concentration at all levels of flow rate (**Figure 2B**), and TP concentration reached a maximum near the combination of 0.63 M NaOH and 0.6 mL/min (**Figure 2C**). Whereas temperature and NaOH concentration had significant positive effects on the extraction of TP, changes in flow rate showed negligible effects on the extraction of TP. The results on the effects of NaOH concentration on the extraction of phenolic compounds were significant ($p < 0.05$) (**Table 4**) and showed that recovery of phenolic compounds increased with increasing concentration of NaOH. This finding is in agreement with published reports showing that NaOH solution is effective in breaking esterified bonds of lignin and can be used for the pretreatment of lignocellulosic material (*32*, *33*). The results of ANOVA and surface response plots showed that, among tested variables, extraction temperature was the most important variable in the extraction of phenolic compounds with PLPW. Data on the stability of phenolic compounds under PLPW water extraction are not available. However, a report on the thermal stability of nine phenolics, including caffeic acid, catechin, *p*-coumaric acid, epicatechin, gentisic acid, and vanillin extracted with pressurized methanol at 150 °C, shows that most of these phenolic compounds tested, except for catechin and epicatechin, are very stable at 150 °C (*34*). In our study, only ferulic acid showed signs of degradation at temperatures above 129.5 °C; all other phenolic compounds were stable at temperatures up to 230.5 °C with 0.63 M NaOH.

The effects of varying all three independent process variables studied are illustrated in a cube plot (**Figure 3**). Each cube corner represents the eight different experimental conditions with the coded levels from −1.68 to +1.68. The highest TP concentration of 5.8 g/kg of DFS was predicted for the combination of a high temperature (A+) and a high concentration of NaOH (B+) with a low flow rate (C−).

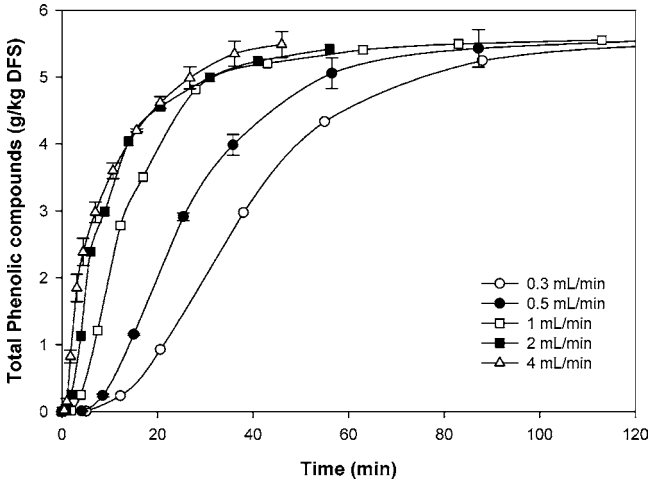

Figure 4. Effect of flow rate on the extraction of TP compounds from 0.85 g of flax shive with PLPW extraction in a 100 mm long × 9 mm i.d. extraction cell at 180 °C, 0.47 M NaOH, and 5.2 MPa.

Thermodynamic and Kinetic Models for PLPW Extraction of Phenolic Compounds. As shown in the ANOVA table (**Table 4**), flow rate had no significant effect on the extraction of phenolic compounds in the range of 0.7−2.3 mL/min when the total volume of NaOH solution used to extract 0.85 g of ground flax shive was 50 mL. Therefore, to verify the effect of flow rate on an extraction rate and evaluate the extraction mechanism model, experimental runs using the "one-factor-at-a-time" method were carried out using the optimum condition from CCD with different flow rates. Ground flax shive (0.85 g) was sequentially extracted with 0.47 M NaOH solution at five different flow rates (0.3, 0.5, 1, 2, and 4 mL/min) at 180 °C. Presumably, the extraction mechanism can be determined by simply comparing the effect of flow rate on the extraction rate of TP (*31*). As presented in **Figure 4**, the extraction of TP profiles shows typical extraction curves for PLPW extraction found in several references (*19*, *31*, *35*).

When the concentration of TP was plotted as a function of time, PLPW extractions yielded 5.4 g/kg of DFS (1 mL/min) and 5.7 g/kg of DFS (0.5 mL/min) at the termination of extraction, and the extraction rates of TP were proportional to the increase in flow rate in the early extraction phase of low flow rates between 0.3 and 2 mL/min. Therefore, we applied the thermodynamic model (eq 4) to the data shown in **Figure 4**. The distribution coefficient (K_D) was calculated from the data at the lowest flow rate of 0.3 mL/min by dividing the concentration of TP in the flax shive after a certain extraction time with the concentration of TP in water during the extraction time (*31*). The obtained K_D value of 30 from 0.3 mL/min was used to calculate predicted curves for other flow rates. When the experimental and the predicted values were compared, the general fits of the experimental curves agreed well with the theoretical curves plotted using the thermodynamic model at flow rate from 0.3 to 1 mL/min (**Figure 5**), thus demonstrating that external mass transfer (elution) controls the PLPW extraction of phenolic compounds from flax shives at low flow rates and that the thermodynamic model is an adequate model for PLPW extraction at low flow rates.

The two-site kinetic model (eq 3) was tested by curve fitting using the Microsoft Excel 2002 solver, and the results are shown in **Figure 6**. The predicted curves were plotted using *F* values, the fraction of analyte released quickly, and determined from extracts collected in the first 10 min of the extractions (**Table 5**). The two-site kinetic model agreed well only with the high flow rates of 2 and 4 mL/min. When the experimental and predicted values at flow rates of 0.3, 0.5, and 1 mL/min were compared, the general fit of the experimental curves did not agree with predicted values, illustrating that the two-site kinetics model is not an adequate model for PLPW extraction at low flow rates.

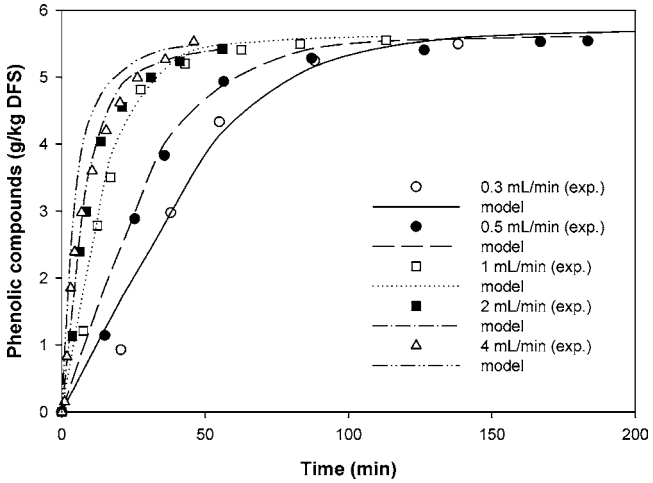

Figure 5. Thermodynamic model fit of PLPW extraction results of the concentration of TP compounds from 0.85 g of ground flax shive with PLPW extraction in a 100 mm long × 9 mm i.d. extraction cell at 180 °C, 0.47 M NaOH, and 5.2 MPa. Symbols represent the experimental date, and the lines are calculated from a thermodynamic model using the K_D value of 30 for all flow rates.

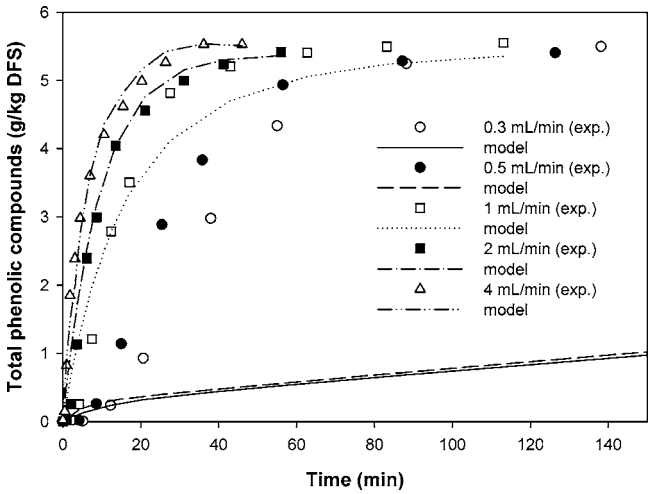

Figure 6. Two-site kinetic model fit of PLPW extraction results of the concentration of TP compounds from 0.85 g of ground flax shive with PLPW extraction in a 100 mm long × 9 mm i.d. extraction cell at 180 °C, 0.47 M NaOH, and 5.2 MPa. Symbols represent the experimental data, and the lines are calculated from a two-site kinetic model using the *F* value calculated at 10 min of each experiment.

Table 5. Comparison of *F*, k_1, and k_2 Value for PLPW Extraction of Phenolic Compounds from Flax Shive Using Two-Site Kinetic Model Calculated by Equation 4

flow rate (mL/min)	*F*	k_1	k_2
0.3	0.041	0.106	0.001
0.5	0.046	0.200	0.001
1.0	0.488	0.096	0.032
2.0	0.709	0.108	0.089
4.0	0.631	0.097	0.099

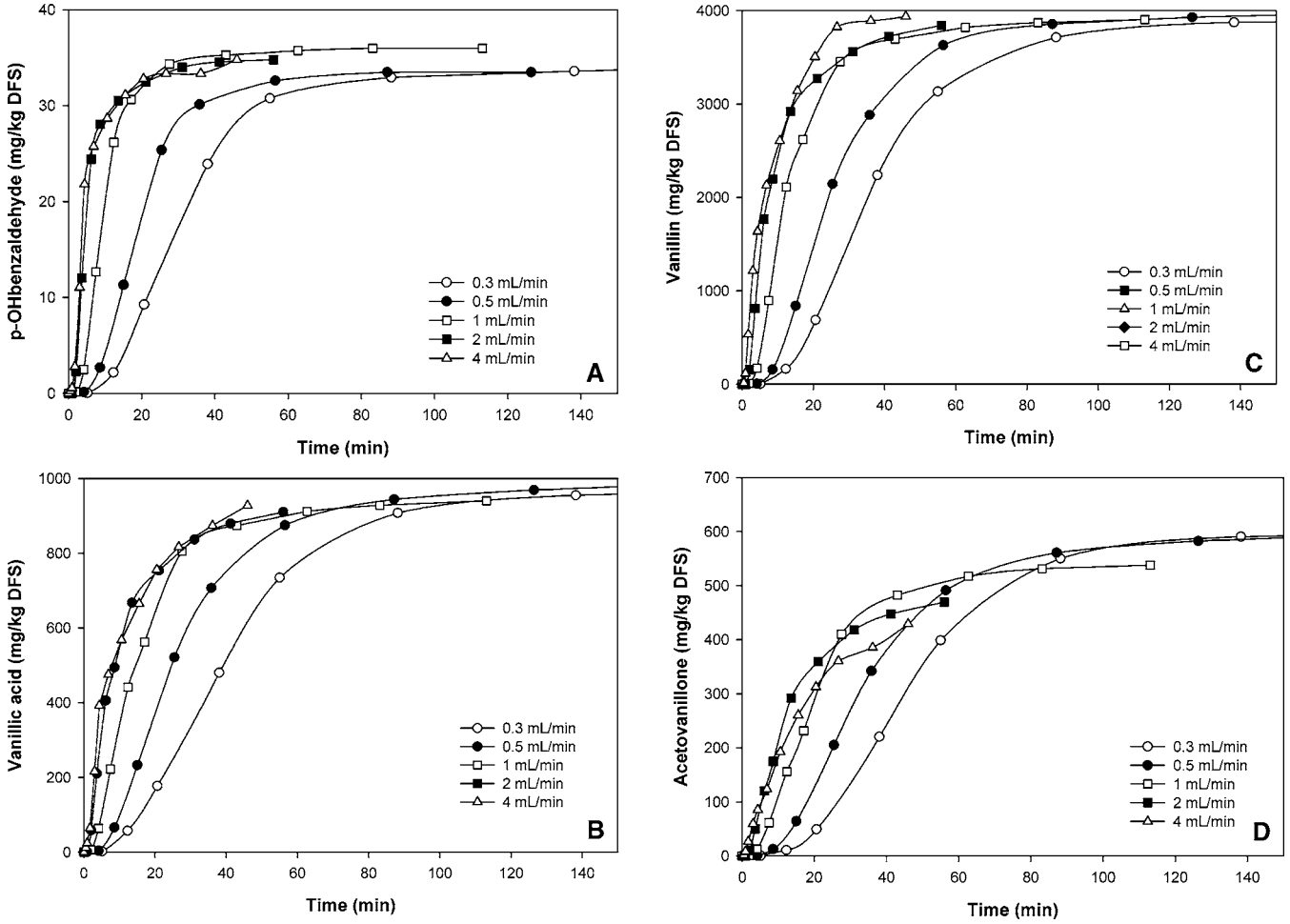

Figure 7. Effect of flow rate on the extraction of (**A**) *p*-hydroxybenzaldehyde, (**B**) vanillic acid, (**C**) vanillin, and (**D**) acetovanillone from 0.85 g of flax shive with PLPW extraction in a 100 mm long × 9 mm i.d. extraction cell at 180 °C, 0.47 M NaOH, and 5.2 MPa.

At the flow rate of 4 mL/min, a total extraction volume of more than 150 mL (water to seed ratio of 176 mL/g) was required to reach equilibrium, whereas the flow rates of 0.3, 0.5, and 1 mL/min required 50 mL of water to reach equilibrium (water to seed ratio of 60 mL/g). Therefore, the use of a low flow rate can increase the extraction of TP with low extraction volume.

Extraction of Phenolic Compounds. The extraction profiles of five main phenolic compounds, including *p*-hydroxybenzaldehyde, vanillic acid, vanillin, and acetovanillone, were plotted as a function of time. Panels **A**, **B**, and **C** of **Figure 7** show that the flow rate substantially enhanced the PLPW extraction rates of *p*-hydroxybenzaldehyde, vanillic acid, and vanillin, respectively, up to 2 mL/min. The extraction time for reaching equilibrium was decreased as flow rate increased, but the increase of flow rate did not change the concentration at equilibrium. Therefore, extraction using low flow rate reduced water consumption and gave high concentration of *p*-hydroxybenzaldehyde, vanillic acid, and vanillin. The extraction pattern of acetovanillone was different from that of the other phenolic compounds (**Figure 7D**). The extraction rate increased with flow rate up to 2 mL/min in the early phase. When the flow rate increased from 0.3 to 4 mL/min, the concentration of acetovanillone decreased from 600 to 350 mg/kg of DFS at equilibrium. In the early extraction phase, the extraction of quickly released acetovanillone on the flax shive surface was governed by the elution. After depletion of quickly released acetovanillone, in the slow extraction phase, the extraction of unreleased acetovanillone was not controlled by the elution, resulting in less impact of flow rate and more dilute extractions at high flow rates.

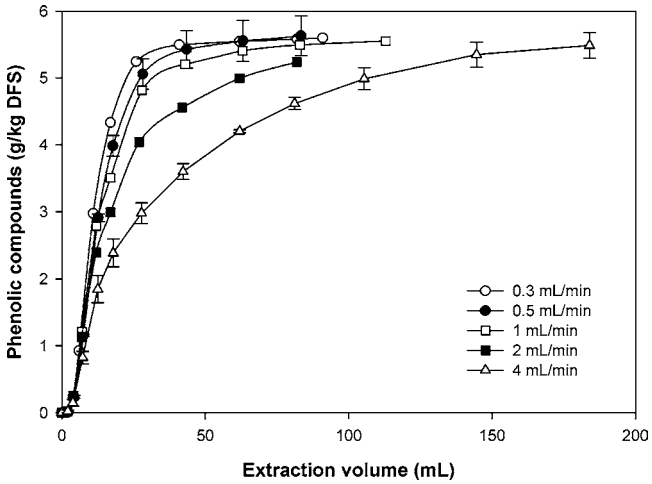

Figure 8. Effect of flow rate on the extraction of TP compounds from 0.85 g of flax shive with PLPW extraction in a 100 mm long × 9 mm i.d. extraction cell at 180 °C, 0.47 M NaOH, and 5.2 MPa.

The yields of TP obtained at different flow rates are plotted against the volume of NaOH solution in **Figure 8**. The equilibrium concentrations of TP were similar to each other. At the flow rates of 0.3, 0.5, and 1 mL/min, a total extraction volume of less than 50 mL (water to seed ratio of 60 mL/g)

was required to reach equilibrium, whereas at 4 mL/min more than 150 mL of NaOH solvent was required to reach equilibrium (water to seed ratio of 180 mL/g). Therefore, the results of the flow rate experiment showed that decreasing flow rate is beneficial to increasing solubilization of all phenolic compounds. The use of a low volume of solvent results in more cost-effective extraction due to high yield of phenolic compounds and decreasing purification cost of phenolic compounds from extract.

Conclusions. PLPW extraction of phenolic compounds, including *p*-hydroxybenzaldehyde, vanillic acid, vanillin, acetovanillone, and ferulic acid, was maximized at the combined conditions of high temperature and high NaOH concentration. When the effects of temperature, NaOH concentration, and flow rate were evaluated by CCD, a maximum TP concentration of 5.8 g/kg of DFS was predicted with a total 50 mL of extract from the combination of high temperature (230.5 °C), high initial concentration of NaOH (0.63 M NaOH), and low flow rate (0.7 mL/min). To validate the model, the predicted values were compared to experimental results. The extraction of phenolic compounds from flax shive at 180 °C, 0.47 M NaOH, and 0.5 mL/min produced a TP concentration of 5.7 g/kg of DFS with 50 mL of extract. The results agreed well with the predicted values from CCD under the same extraction conditions. The PLPW extraction mechanism was also investigated, and it was found that it could be described by a thermodynamic model at high flow rate and a two-kinetic model at low flow rate. Further research, aimed at optimizing the geometric variables of the extraction vessel, equipment design, and pretreatment of flax shive, is in progress in our laboratory.

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