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Recovery of Isoflavone Aglycones from Soy Whey Wastewater Using Foam Fractionation and Acidic Hydrolysis

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ABSTRACT: The purpose of this work was to recover isoflavone aglycones from industrial soy whey wastewater, where the isoflavone aglycones mainly existed in the form of β-glycosides. First, foam fractionation was used for effectively concentrating the total soy isoflavones, including isoflavone aglycones and β-glycosides, from the wastewater. Fourier transform infrared spectroscopy indicated the existence of complexes of soy isoflavones and soy proteins. When soy proteins were used as the collectors, a high enrichment ratio of 3.68 was obtained under the optimal operating conditions of temperature of 50 °C, pH of 5.0, volumetric air flow rate of 100 mL/min, and loading liquid height of 400 mm. Subsequently, acidic hydrolysis was used for hydrolyzing β-glycosides in the foamate into aglycones. Using response surface methodology, a hydrolytic percentage could reach 96% under the optimum hydrolysis conditions of hydrolytic temperature of 80 °C, hydrochloric acid concentration of 1.37 mol/L, and hydrolytic time of 90 min.

KEYWORDS: *Isoflavone aglycones, β-glycosides, wastewater, foam fractionation, acidic hydrolysis*

INTRODUCTION

Soy proteins are massively consumed as indispensable daily foods because of their high nutrition and their effective prevention of hormone-dependent cancers, osteoporosis, and menopausal symptoms.[1−3] Much evidence has indicated that the medicinal importance of soy proteins is mainly attributed to a group of polyphenolic secondary metabolites, soy isoflavones, which readily bond with proteins.[4,5] The major soy isoflavones consist of three isoflavone aglycones (i.e., daidzein, genistein, and glycitein) and their glycosides (i.e., acetyl-, malonyl-, and β-glycosides).[6] They have significant pharmacological actions because of their similar structures to that of estrogen.[7] Therefore, soy isoflavones have gained increasing interest as healthcare products.

Soy isoflavones are mostly dissolved in soy whey wastewater generated during isoelectric precipitation of soy protein isolate (SPI).[8] However, most plants discharge large quantities of the wastewater to a sewage treatment plant to translate its useful compounds into sludge, and this results in a heavy loss of the high-value compounds. For reusing soy isoflavones, the current work is purposed to recover soy isoflavones from actual soy whey wastewater. This investigation can also decrease the cost for the wastewater treatment. However, acetyl-glycosides and malonyl-glycosides are hydrolyzed into β-glycosides, which cannot be directly used by the human body during SPI production.[6] In addition, antioxidant activity and bioavailability of isoflavone aglycones are the highest among all of the soy isoflavones.[9] Thus, a further hydrolyzation is indispensible to obtain isoflavone aglycones from the separated products.

Soy isoflavones are generally separated from soybean seeds using solvent extraction or column chromatography.[10,11] However, both of the methods cannot be used to recover isoflavone aglycones from soy whey wastewater because the wastewater has a low isoflavone aglycone concentration but a huge discharge. In this work, foam fractionation will be adopted as a promising separation technique for effective recovery of isoflavone aglycones. It is commonly used as an effective pretreatment step for concentrating or removing surface-active compounds from a dilute aqueous solution, because of its simple equipment, low investment, low energy consumption, and environmental compatibility.[12,13] Moreover, some non-surface-active compounds (i.e., dye and metal ion) can also be separated by foam fractionation using the surfactant as a collector.[14] The hydrophobicity of polyphenols is weak and, thus, cannot be adsorbed on the bubble surface.[15] However, proteins and polyphenols can form complexes with hydrogen bonding, ionic or hydrophobic interactions,[16] and a lot of soy proteins, which have relatively high surface activity and are also dissolved in soy whey wastewater.[17] Thus, it is feasible to recover polyphenols from the wastewater by foam fractionation with soy proteins as the collectors.

In the present work, the protein−isoflavone complexes in soy whey wastewater will be first determined by Fourier transform infrared spectroscopy (FTIR). On this foundation, foam fractionation of the total soy isoflavones will be investigated at various temperatures, pH values, and volumetric air flow rates. Then, the foam fractionation products, i.e., the foamate, will be used as the raw material for obtaining isoflavone aglycones by acidic hydrolysis,[18] and the efficiencies will be examined using response surface methodology (RSM). All of the efforts are aimed at effectively recovering isoflavone aglycones from soy whey wastewater and supplying a new method for comprehensive use of the industrial wastewater.

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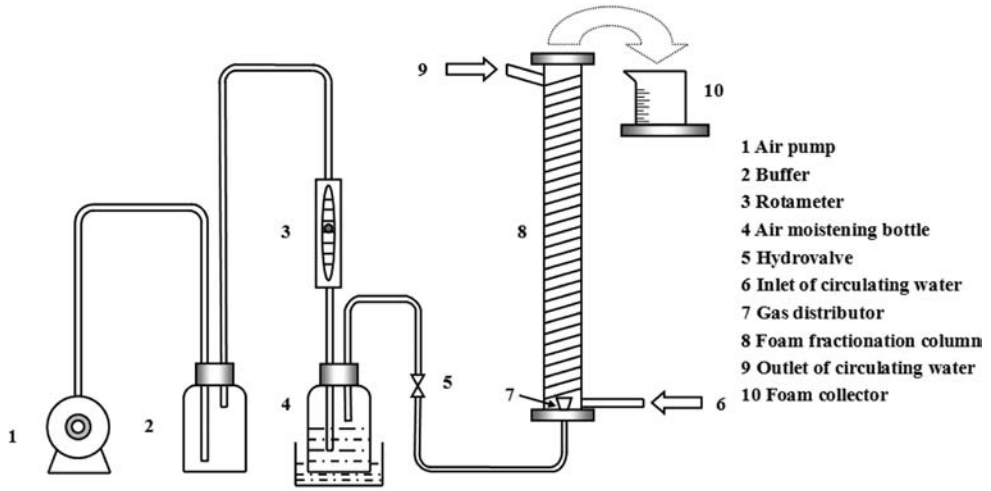

Figure 1. Schematic diagram of the experimental setup.

MATERIALS AND METHODS

Materials and Reagents. Soy whey wastewater was provided by Xiang Chi Soy Protein Industry Co., Ltd., Shandong, China. It had a chemical oxygen demand (COD) of 10 000 mg/L, biochemical oxygen demand (BOD) of 8000 mg/L, total nitrogen (TN) of 1500 mg/L, proteins of 4000 mg/L, soy isoflavones of 400 mg/L, and pH of 4.3. The standard isoflavones (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) and the standard soy proteins were purchased from Sigma Chemical Co., St. Louis, MO. Ethanol was used as the extracting solvent. Acetonitrile and acetic acid were used as the mobile phase for high-performance liquid chromatography (HPLC) analysis. Hydrochloric acid was used in the acidic hydrolysis experiments. All of the solvents and reagents were purchased from Fengchuan Chemical Reagents Co., Ltd., Tianjin, China.

HPLC Identification of Soy Isoflavones. Soy whey wastewater of 5 mL was filtered using a 0.45 μm membrane filter to remove undissolved materials. The filtrate was freeze-dried (Eyela Fdu-1200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and the resultant powder was extracted with 10 mL of 80% ethanol using ultrasonic concussion for 20 min and centrifuged (4500 rpm for 10 min). The supernatant was filtrated using a 0.45 μm membrane filter to serve as the sample for HPLC analysis. On the basis of the research by Nara et al.,[7] the sample was analyzed using a diode array detector (Agilent Technologies, Inc., Santa Clara, CA) and a Symmetry C_{18} (250 × 4.6 mm, 5 μm, Shimadzu, Japan) with 0.1% acetic acid/acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min and column temperature of 40 °C. The sample was measured by an ultraviolet (UV) detector at the maximal absorption wavelength of 260 nm. The total run time was 22 min.

FTIR Determination of the Protein−Isoflavone Complexes. Soy whey wastewater of 25 mL was filtered using a 0.45 μm membrane filter to remove undissolved materials. The filtrate was freeze-dried to collect the resultant powder as the sample to be tested. Moreover, genistein (0.4 g) and the soy proteins (1.0 g) were mixed thoroughly to serve as the sample of the physical mixture. The FTIR spectra of genistein, the soy proteins, the sample of the physical mixture, and the sample of soy whey wastewater were collected between 4000 and 400 cm^{-1} on a Vector-22 infrared spectrophotometer (Bruker, Germany) with 256 scans at a resolution of 4 cm^{-1} using the KBr method. The data were recorded and processed using Opus software (Bruker, Germany).

Recovery of Soy Isoflavones from Soy Whey Wastewater Using Foam Fractionation. Figure 1 presents a schematic diagram of the experimental setup. The foam fractionation column was constructed of a polymethyl methacrylate tube with an inner diameter of 44 mm, and its length was 1200 mm. A porous polyethylene membrane with a pore diameter of 250 μm was mounted at the bottom of the column to serve as a gas distributor. In each experiment, a certain volume of soy whey wastewater was loaded into the foam fractionation column and then air was sparged through the gas distributor to form numerous bubbles in the loading wastewater. Proteins and their complexes with soy isoflavones adsorbed on the bubble surfaces in the bulk liquid to form stable foam when the bubbles emerged from the liquid−foam interface. With the foam rising, the interstitial liquid drained out of it and returned the bulk liquid due to gravity. The foam would become greatly drier when it was discharged out of the column; therefore, soy isoflavones would be effectively concentrated.

The performance parameters of foam fractionation were defined as the enrichment ratio (E) and recovery percentage (R)

$$E = \frac{C_0V_0 - C_wV_w}{C_0V_f} \quad (1)$$

$$R = \frac{C_0V_0 - C_wV_w}{C_0V_0} \times 100\% \quad (2)$$

where C_o and C_w are the total soy isoflavone concentrations in the feeding solution and the residual solution (mg/L), respectively, and V_o, V_f, and V_w are the volumes (L) of the feeding solution, the foamate, and the residual solution, respectively.

Measurement of Foam Stability. The stability of the foam formed by soy whey wastewater was determined using the Ross−Miles method.[19] The wastewater of 200 mL was fed into a scaled pipet with an orifice of 2.9 mm in internal diameter and 10 mm in length. The wastewater in the pipet was allowed to fall from a height of 900 mm onto 50 mL of the wastewater loaded in a cylindrical column of 50 mm in internal diameter. The column was surrounded by a water jacket to control the foam temperature. The stability of the foam could be defined as its half-collapse time ($time_{1/2}$, s).

Measurement of Viscosity. Viscosity of soy whey wastewater was measured using a modified suspended-level Ubbelohde viscometer. The apparatus was submerged in a thermostatic bath with a deviation of ±0.05 °C to control its temperature. The viscosity of liquid vertically flowing down in a capillary due to gravity could be calculated by Poiseuille law,[20] which is expressed as follows:

$$\frac{\eta}{\rho} = At - \frac{B}{t} \quad (3)$$

where η and ρ are viscosity (mPa/s) and density (g/cm^{-3}), respectively, t is the flow time, and A and B are constants. The density ρ was measured using a vibrating-tube digital densimeter (Anton Paar, DMA 60/602).[21] The flow time for a given volume of liquid flowing through the capillary was measured with an accurate stop watch with a resolution of 0.1 s. If the flow time was more than

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100 s, the term of B/t could be neglected because B was less than 1. Therefore, eq 3 was reduced to linear eq 4.

$$\eta = A\rho t \quad (4)$$

Therefore, the viscosity η was obtained by comparing it to that of pure water, which could be checked from a handbook of chemical technology.

RESULTS AND DISCUSSION

HPLC Analysis for Soy Isoflavones in Soy Whey Wastewater. The HPLC chromatogram of the soy whey wastewater sample in Figure 2 showed the peaks of daidzin, glycitin, genistin, daidzein, and genistein had the highest content. The results indicated that β-glycosides (daidzin, glycitin, and genistin) were the major soy isoflavones in the wastewater and the contents of their aglycones (daidzein and genistein) were very low. It was also suggested that acetyl-glycosides and malonyl-glycosides were adequately hydrolyzed to β-glycosides during SPI production. Therefore, an effective hydrolytic step would be necessary for obtaining the soy isoflavone aglycones.

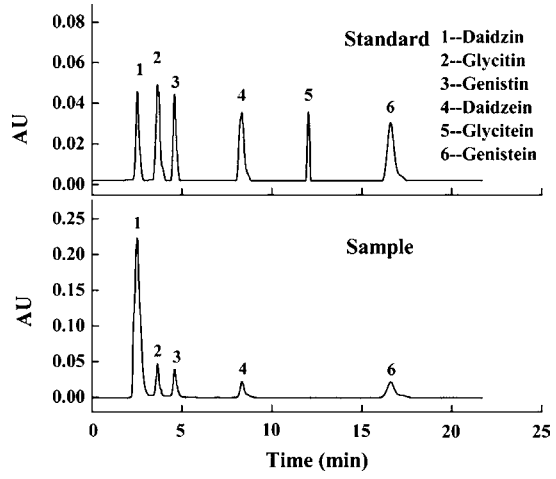

Figure 2. HPLC chromatogram of soy isoflavones in soy whey wastewater.

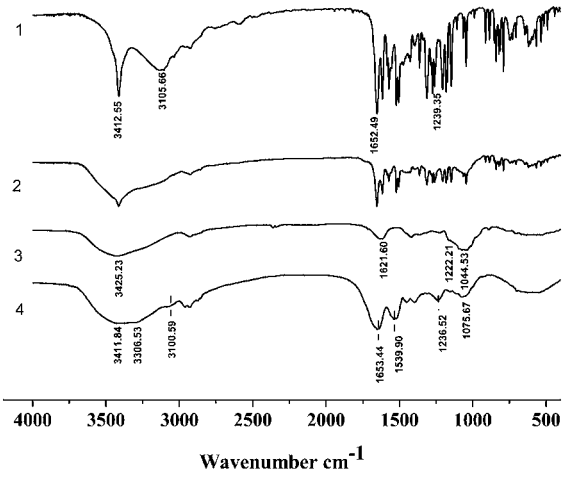

Figure 3. FTIR spectra of (1) genistein, (2) the sample of the physical mixture, (3) the soy proteins, and (4) the sample of soy whey wastewater.

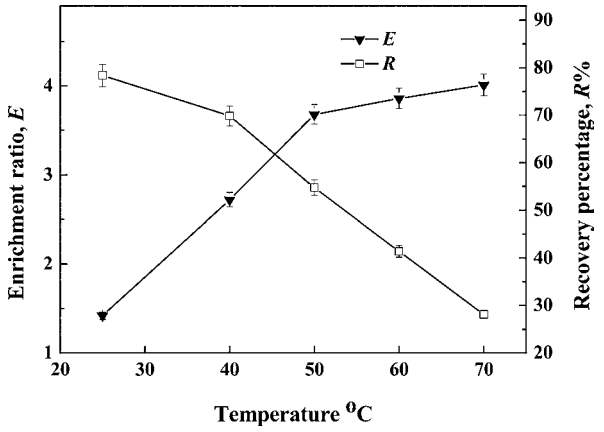

Figure 4. Effects of the temperature on E and R.

Table 1. Effects of the Temperature on the Viscosity and Foam Stability of Soy Whey Wastewater

temperature (°C)	viscosity (mPa/s)	$time_{1/2}$ (s)	operation time (min)
25	1.20 ± 0.02	256 ± 0.2	227 ± 2
30	1.06 ± 0.01	206 ± 0.2	206 ± 2
40	0.847 ± 0.01	125 ± 0.2	163 ± 2
50	0.712 ± 0.01	80 ± 0.2	110 ± 2
60	0.623 ± 0.02	53 ± 0.2	73 ± 2
70	0.489 ± 0.01	17 ± 0.2	44 ± 2

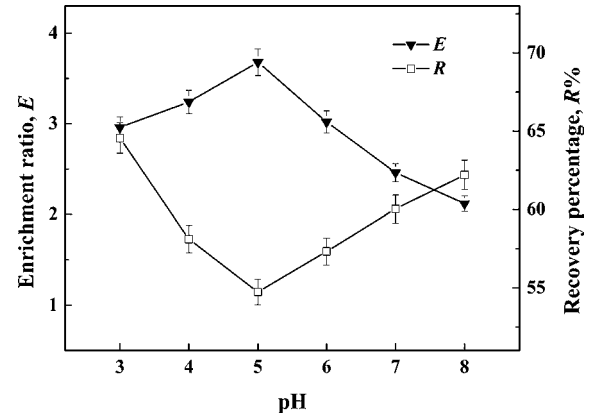

Figure 5. Effects of the pH on E and R.

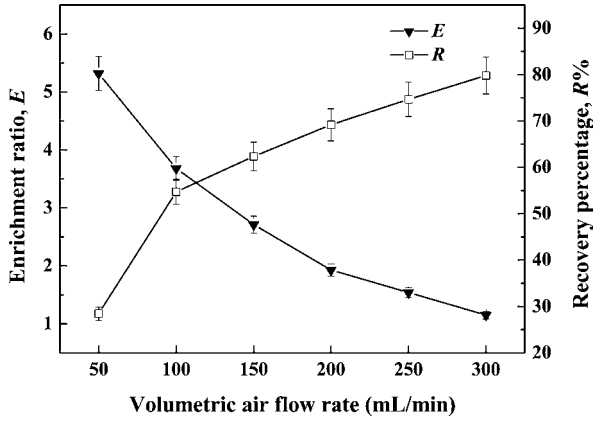

Figure 6. Effects of the volumetric air flow rate on E and R.

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Table 2. Experimental Design of Five-Level and Three-Variable CCD

test set	X_1, hydrochloric temperature (°C)	X_2, hydrochloric time (min)	X_3, hydrochloric acid concentration (mol/L)	GHP (%) actual value	GHP (%) predicted value
1	80	60	0.32	64.39	70.41
2	80	9.55	2.00	90.15	86.87
3	96.82	60	2.00	83.33	72.75
4	70	30	1.00	59.24	53.31
5	63.18	60	2.00	54.74	56.07
6	80	60	2.00	94.83	95.39
7	80	110.45	2.00	96.92	90.95
8	80	60	2.00	95.39	95.39
9	80	60	2.00	94.96	95.39
10	90	30	1.00	73.62	76.01
11	70	30	3.00	57.91	64.24
12	70	90	1.00	86.76	85.27
13	90	90	1.00	87.41	87.62
14	80	60	2.00	95.27	95.39
15	80	60	2.00	95.43	95.39
16	80	60	2.00	94.89	95.39
17	90	30	3.00	73.69	81.72
18	90	90	3.00	42.14	54.61
19	80	60	3.68	67.11	51.84
20	70	90	3.00	53.32	57.47

FTIR Analysis for the Protein−Isoflavone Complexes. The FTIR spectra of genistein, the soy proteins, the sample of the physical mixture, and the sample of soy whey wastewater are showed in Figure 3. The FTIR spectrum of genistein showed the prominent absorption bands at ∼3412.55 cm^{-1} (for O−H), ∼3105.66 cm^{-1} (for Ar−H), ∼1652.49 cm^{-1} (for the aromatic conjugated C═O), ∼1239.35 cm^{-1} (for Ar−OH), and ∼1044.42 cm^{-1} (for C−O). The prominent absorption bands of the soy proteins were shown at ∼3425.23 cm^{-1} (for O−H), ∼1621.60 cm^{-1} (for C═O), ∼1222.21 cm^{-1} (for C−N), and ∼1200−1000 cm^{-1} (for C−O). Apparently, the spectrum of the physical mixture of the soy proteins and genistein displayed a simple spectral addition effect of the spectra of the two compounds. However, in comparison to the spectrum of the soy proteins, several characteristic absorption bands appeared in the spectrum of the whey wastewater, such as ∼3306.53 and ∼1539.90 cm^{-1} (for N−H), ∼3100.59 cm^{-1} (for Ar−H), and ∼1236.52 cm^{-1} (for Ar−OH). Additionally, the peak shape and the intensity of C═O (∼1653.44 cm^{-1}) and C−O (∼1075.67 cm^{-1}) were also changed. These results might have resulted from the carboxylic ester, which linked soy proteins and soy isoflavones, because the stretching vibration of C═O was more intense than that of C−O in the ester group. Thus, the protein−isoflavone complexes did exist in the wastewater, and the esterification was the main linkage between soy proteins and soy isoflavones.

Recovery of Soy Isoflavones from Soy Whey Wastewater Using Foam Fractionation. Foam fractionation of the total soy isoflavones (involving isoflavone aglycones and β-glycosides) from soy whey wastewater was investigated in this section. The effects of the temperature, pH, and volumetric air flow rate were studied for optimizing the operating conditions to obtain a high enrichment ratio.

Effects of the Temperature on E and R. The temperature is an important parameter that can significantly decrease the viscosity of a solution and, thereby, affect the liquid fluidity in foam.[22] The effects of the temperature on E and R were studied under the conditions of liquid loading height of 400 mm, volumetric air flow rate of 100 mL/min, and pH of 5.0. The temperature ranged from 25 to 70 °C. The results are presented in Figure 4.

From Figure 4, the enrichment ratio of soy isoflavones increased from 1.42 to 4.51, while the recovery percentage accordingly decreased from 78.36 to 28.14% with an increasing temperature from 25 to 70 °C. This trend could be explained from the two following aspects. On the one hand, the increase of the temperature could intensify molecular thermal motion. The adsorption of the protein−isoflavone complexes on the bubble surfaces could be improved with the bubbles rising through the bulk liquid. On the other hand, the increased temperature could decrease the viscosity of the solution, as presented in Table 1. Thus, foam drainage was enhanced. As a result, the enrichment ratio was effectively improved.

However, the protein is sensitive to the temperature because a high temperature can result in denaturation, aggregation, or gelation,[23] thus decreasing its foam stability. From Table 1, the half collapse time ($time_{1/2}$, s) decreased with an increasing temperature and, hence, the operation time also decreased. When the temperature was higher than 50 °C, the enrichment ratio increased slowly and the recovery percentage greatly decreased because it was difficult for the wastewater to form stable foam. Therefore, to effectively concentrate both soy proteins[24] and soy isoflavones, foam fractionation should be operated under the appropriate temperature. With all of the above considered, 50 °C was selected as the optimal temperature.

Effects of pH on E and R. The pH of a solution affects molecular net charge, interfacial tension, and bubble size in foam fractionation, especially for the proteins.[25] The effects of pH on E and R were studied under the conditions of temperature of 50 °C, loading liquid height of 400 mm, and volumetric air flow rate of 100 mL/min. pH ranged from 3.0 to 8.0. The results are presented in Figure 5.

As shown in Figure 5, with the increase of pH from 3.0 to 8.0, the enrichment ratio of the soy isoflavones increased from 2.76 to 3.68 and then decreased to 2.12, while the recovery percentage decreased from 64.56 to 54.74% and then increased to 62.21%. The maximum enrichment ratio of 3.68 was obtained at pH 5.0.

Soy isoflavones have no surface activity, but they can adsorb on the bubble surface by interacting with soy proteins. In addition, proteins are amphoteric molecules, and their properties are greatly affected by pH. Thus, the adsorptive properties of the protein−isoflavone complexes closely depended upon pH. In general, the surface excess of a protein is the highest at its isoelectric point because the intermolecular repulsion is the smallest.[26] It was reported that soy proteins were a group of proteins of which isoelectric points were in the range of pH 4.5−5.7.[27] In addition, the foam film thickness became thinner at the isoelectric point, and this could increase the average bubble diameter.[28] Resultantly, bubble coalescence was enhanced, and the maximum enrichment ratio was obtained at pH 5.0. Moreover, the soy isoflavones existed in the solution as neutral molecules at acidic conditions and then partially transformed into anionic forms with increasing pH.[29] On the basis of the above results, pH 5.0 was chosen as the optimal initial pH.

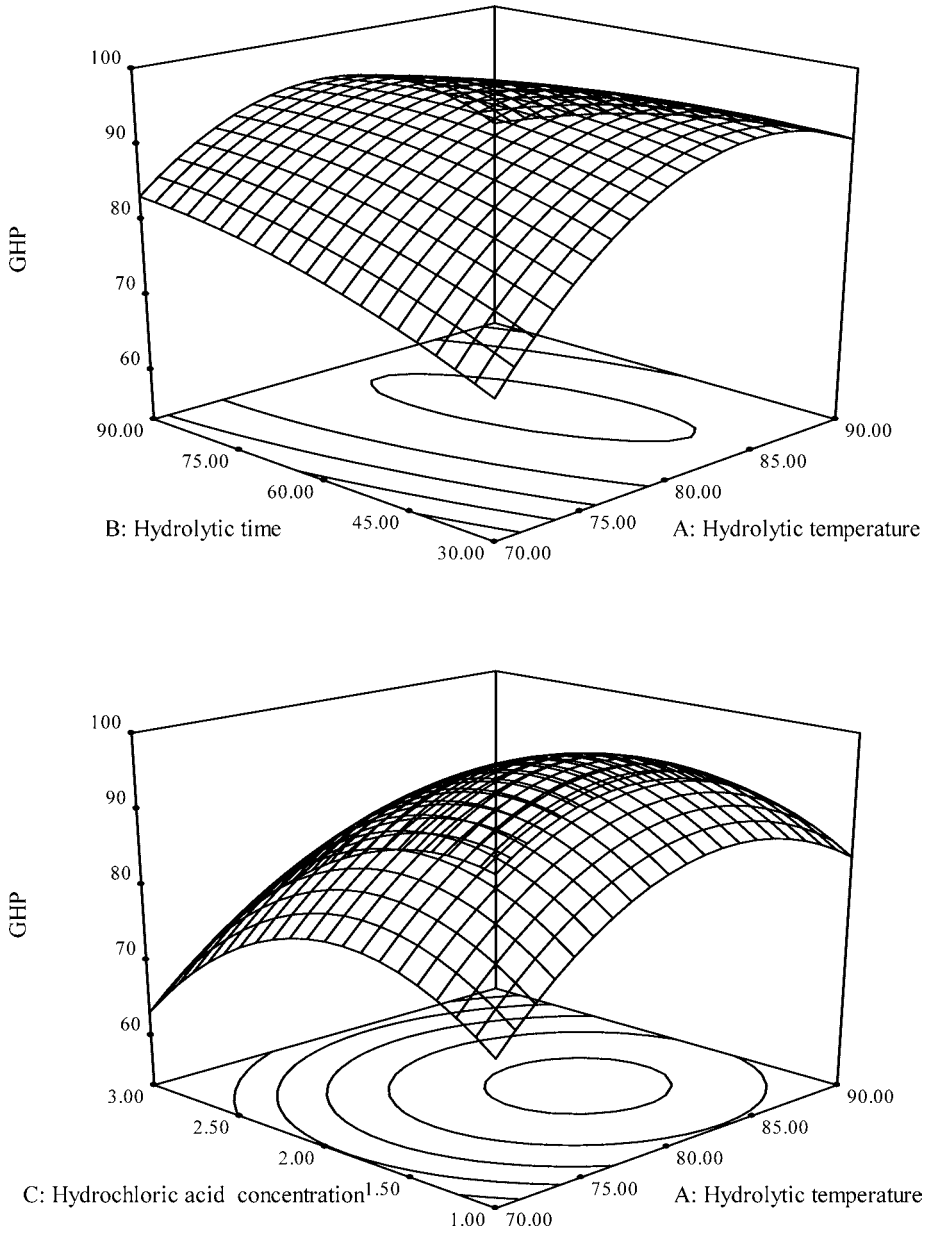

Figure 7. Response surface graphs for the effects of hydrolytic temperature, hydrolytic time, and hydrochloric acid concentration on GHP.

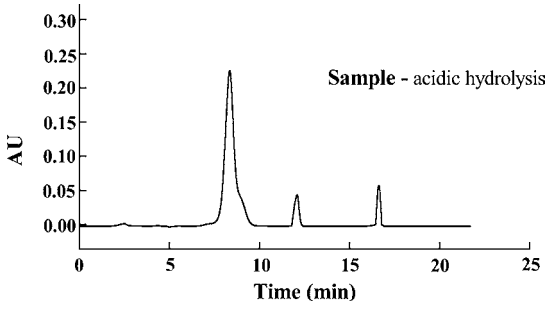

Figure 8. HPLC chromatogram of soy isoflavones in the foamate by acidic hydrolysis.

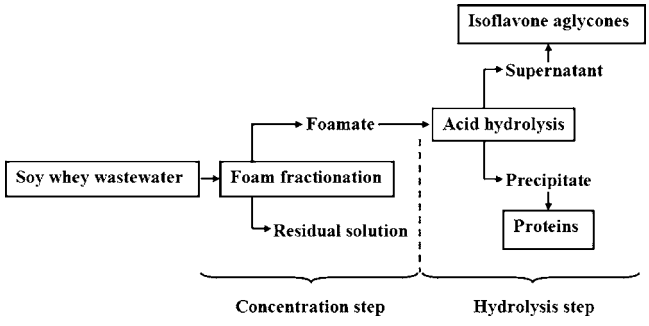

Figure 9. Schematic diagram of foam fractionation and acidic hydrolysis.

Effects of the Volumetric Air Flow Rate on E and R. The volumetric air flow rate affects the residence time of bubbles in the bulk liquid.[30] The effects of the volumetric air flow rate on *E* and *R* were studied under the conditions of temperature of 50 °C, pH of 5.0, and loading liquid height of 400 mm. The volumetric air flow rate ranged from 50 to 300 mL/min. The results are shown in Figure 6.

As shown in Figure 6, with the increase of the volumetric air flow rate from 50 to 300 mL/min, the enrichment ratio of soy

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isoflavones decreased from 5.32 to 1.15, while the recovery percentage increased from 28.45 to 79.80%. At a low volumetric air flow rate, the residence time of bubbles in the bulk liquid was longer and, thereby, the surface excess of the protein−isoflavone complexes would be improved. In addition, the low air flow rate also contributed to the drier foam.[31] Thus, the enrichment ratio was high. With an increasing volumetric air flow rate, the surface excess of the protein−isoflavone complexes would be decreased by the decreased bubble residence time. The foam would also become wetter because the time for foam drainage was shortened. Therefore, the enrichment ratio of soy isoflavoines was low, while the recovery percentage was high, at a high volumetric air flow rate. Considering the above results, 100 mL/min was selected as the optimal volumetric air flow rate.

On the basis of the results above, with the loading liquid height fixed at 400 mm, the total soy isoflavones could be effectively concentrated from soy whey wastewater under the optimal operating conditions of temperature of 50 °C, pH of 5.0, and volumetric air flow rate of 100 mL/min. Under these conditions, a higher enrichment ratio of 3.68 was obtained with a recovery percentage of 54.74%, and the concentration of soy isoflavones in the foamate was increased from 400 to 1472 mg/L. Therefore, the foamate could be used as the raw material to further hydrolyze for obtaining the isoflavone aglycones.

Acidic Hydrolysis. Acidic hydrolysis of β-glycosides into aglycones was studied in this work because of its outstanding advantages, involving low cost, simple technology, easy control, and high hydrolytic percentage.[32] The foamate from foam fractionation was used as the raw material. The operating parameters of hydrochloric acid concentration, hydrolytic time, and hydrolytic temperature were optimized by using RSM. The experiments were conducted using a five-level, three-variable central composite orthogonal and rotatable design (CCD) with six replicated at the central point. The 20 tests were set using the software Design-Expert 8.05. The polynomial model of response surfaces was fitted to the response variable, namely, glycoside hydrolytic percentage (GHP, %). Statistical analysis of the experimental data was performed by RSM using the same software.

Table 2 shows the experimental conditions and the results of acidic hydrolysis according to the factorial design. Multiple regression analysis was performed on the basis of the experimental data, and the coefficients of the model were evaluated for significance. The regression model was significant at the considered confidence level because the p value was less than 0.0001. The predictive equation of the regression model for hydrolyzing glycosides into aglycones is presented in eq 5. The surface response graphs of the interactions between hydrochloric acid concentration, hydrolytic time, and hydrolytic temperature are shown in Figure 7.

$$\begin{aligned}\mathrm{GHP}\ (\%) = {} & 95.39 + 4.96X_1 - 1.21X_2 - 5.52X_3 \\ & - 5.09X_1X_2 - 1.30X_1X_3 - 9.68X_2X_3 \\ & - 10.95X_1^2 - 2.29X_2^2 - 12.12X_3^2 \end{aligned} \quad (5)$$

From Figure 7, with the increase of the hydrolytic temperature, hydrochloric acid concentration, and hydrochloric time, the GHP first increased and then decreased. The concentration of hydrochloric acid should be kept at dilute acid because the structures of isoflavone aglycones might be destroyed in the condition of concentrated acid. The increase of the temperature could contribute to accelerating the hydrolytic rate. However, acidic hydrolysis at high temperatures resulted in a significant degradation of genistein.[33] Thus, the hydrolytic temperature should be lower than 90 °C. The optimal operating conditions obtained using the model were as follows: hydrolytic temperature of 80 °C, hydrochloric acid concentration of 1.37 mol/L, and hydrolytic time of 90 min, where the maximum response value of 99% was predicted. Under the optimal conditions, a mean experimental response value of 96% was obtained. Additionally, the HPLC chromatogram of the foamate after acidic hydrolysis is presented in Figure 8. Therefore, the acidic hydrolysis could effectively hydrolyze β-glycosides into aglycones.

In this work, foam fractionation and acidic hydrolysis were used for recovering isoflavone aglycones from soy whey wastewater. The schematic diagram is presented in Figure 9. During the acidic hydrolysis process, most soy proteins in the foamate were denatured to form precipitate, which was removed by centrifugation, and isoflavone aglycones existed in the supernate. Under the optimal operating conditions, the enrichment ratio and the glycosides hydrolytic percentage could reach 3.68 and 96%, respectively. Finally, the total concentration of isoflavone aglycones in the supernate reached as high as 1400 mg/L. In the future work, the supernate will be further treated by resin adsorption or ionic-liquid-based extraction to obtain the product of high-purity isoflavone aglycones.[34,35] Furthermore, the product will also be analyzed to determine its components, and then its medicinal importance will be evaluated.

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Notes

The authors declare no competing financial interest.

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