Ethanol Extraction of Polyphenols in an Immersion Extractor. Effect of Pulsing Flow

J. Sineiro, H. Domínguez, M.J. Núñez*, and J.M. Lema

Department of Chemical Engineering, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain

ABSTRACT: A comparative study on polyphenol extraction from sunflower press cake in a semicontinuous pulsed-flow immersion extractor and in a conventional laboratory immersion extractor was developed. The solvent was 96% (vol/vol) ethanol. No difference in the residual polyphenol content in the cake was observed at short times, but after 10 h, the pulsed extractor showed a higher polyphenol concentration in the outlet miscella. In addition, the effective diffusivity of polyphenols in sunflower press cake was estimated.

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KEY WORDS: Diffusivity, extraction, polyphenols, pulsed extractor.

Phenolic compounds account for 2–3% of sunflower meal. Chlorogenic acid (3-{[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy}-1,4,5 trihydroxycyclohexane-carboxylic acid) is the major phenolic compound in sunflower seeds (1), is mainly located in the aleurone or protoplasts, and is linked to the 11S sunflower storage protein by hydrogen bonding (2). The presence of polyphenols in the meals obtained from industrially extracted sunflower provides green-colored protein products because of polyphenols oxidation to quinones at the pH values employed to solubilize the protein (3). Moreover, the resulting quinones are susceptible to reaction with the proteins, by covalent bonding to amino or thiol groups; this reduces the nutritional value and stability of the product and alters its functional properties and organoleptic characteristics (4).

Several methods have been described that efficiently extract polyphenols from sunflower meal to yield a protein-rich product free from these antinutritional factors (3,5–8). Ethanol is a good solvent for polyphenols at moderate temperatures (6,9); it is also an alternative solvent to hexane for oil extraction, for which recent interest has been generated because of safety, environmental, and health concerns.

This paper deals with the extraction of polyphenols from semi-defatted sunflower press cake. Despite the fact that the few studies on the extraction kinetics of polyphenols (10) have been carried out on finely ground material, it is interesting to carry out similar studies on extruded material from actual industrial processes. This material allows the use of a packed bed, with less risk of channeling than with ground material. The aim of this study was to compare the performance of a laboratory immersion extractor, operating at continuous flow, with a pulsed extractor, because the application of pulsed flow may alter the packing and reduce the tendency of the solvent to channel in the packed bed.

MATERIALS AND METHODS

Partially dehulled sunflower seeds were kindly supplied by ALCO, S.A. (Maia, Portugal). These seeds were prepressed in a laboratory screw press operating between 85 and 92°C, to obtain a semi-defatted cake; the characteristics are summarized in Table 1. The extraction of polyphenols from the cake was carried out with reagent-grade 96% (vol/vol) ethanol (Analema, Vigo, Spain).

Batch extraction. Extractable polyphenols from the semi-defatted press cake were determined by batch extracting 2.757 g sunflower cake five times with 50 mL 96% ethanol. Each extraction stage was performed in stoppered Erlenmeyer flasks for 24 h in a rotary shaker at 150 rpm and 50°C. The last contact was prolonged for 48 h. The suspension was sedimented, and the sediment was recovered and submitted to the next extraction stage. The liquid phase was analyzed for polyphenol concentration.

TABLE 1
Characteristics of the Extractors and the Cake

Packed-bed volume	47.7 cm^3
Extractor void space	31.8 cm^3
Residence time in the packed bed	540 s
Residence time in the liquid over the bed	600 s
Feed flow	0.048 mL · s^{-1}
Bed void volume	54.3%
Temperature	50°C
Pulsed extractor	
Pulsing frequency	0.032 Hz
Amplitude	0.0786 cm
Sunflower cake	
Sunflower cake in each bed	17 g
Cake moisture content	4.76% (dry basis)
Cake oil content	32.73% (dry basis)
Cake flake thickness	0.6 mm

*To whom correspondence should be addressed.

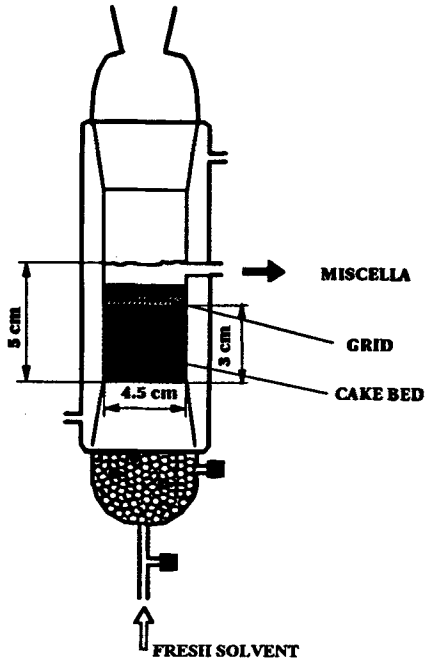

FIG. 1. Laboratory immersion extractor.

Column extraction. Sunflower cakes were extracted in laboratory immersion extractors of 4.5 cm i.d. and 10 cm height. The extractors were kept at 50°C by a thermostated external water bath. A condenser was fitted to avoid solvent losses. Extraction was accomplished by pumping fresh solvent through the cake bed. The solvent was pumped upward from the bottom, where a bed of glass spheres shared the flow. The outlet miscella was sampled periodically, and the polyphenol concentration was recorded. One of the extractors operated with continuous flow and the other with pulsed flow; the pulsed flow was achieved by means of a flexible tube, in which the solvent accumulated until an electrical valve was opened. A timer was used to control the opening frequency of the pulsing device (11). The scheme of the immersion extractor appears in Figure 1, and the experimental set-up is shown in Figure 2. The operational characteristics are shown in Table 1. The bed void volume, corresponding to the initial value, was calculated from the residence time in the bed and the solvent flow during the initial filling of the extractor.

The concentration of polyphenols, expressed as chlorogenic acid concentration, in the ethanolic miscella was determined by ultraviolet absorbance at 330 nm with a Hitachi U-2000 UV-VIS spectrophotometer (Hitachi Ltd., Tokyo, Japan). Analytical-grade chlorogenic acid (Sigma-Aldrich Química, Madrid, Spain) was used as the standard for a calibration graph prepared from a stock solution in ethanol (15 mg/L, its absorbance being 0.680).

RESULTS AND DISCUSSION

The results of the batch extraction are shown in Table 2. Extracted polyphenols were calculated from the measured polyphenol concentrations in the miscella, C_{Et} (mg/L ethanol):

$$\text{extracted polyphenols} = \sum_{1}^{n} C_{Et} \cdot V \qquad [1]$$

where n = number of extractions, and V = volume of ethanol in each extraction stage (L).

To obtain white or creamy protein isolates at pH 9 or 10 (2,6,11,12), the polyphenol content must be reduced to 0.3 g/100 g meal. In the cakes used in this work, it is necessary to reduce the residual polyphenol content to 11.8% of the initial content, which requires at least four extractions with fresh ethanol.

The results for the experimental polyphenol concentration (C_{Et}) in the outlet miscella from the semicontinuous extraction are shown in Figure 3. Although differences in the graph between the pulsed and nonpulsed extractions are not large,

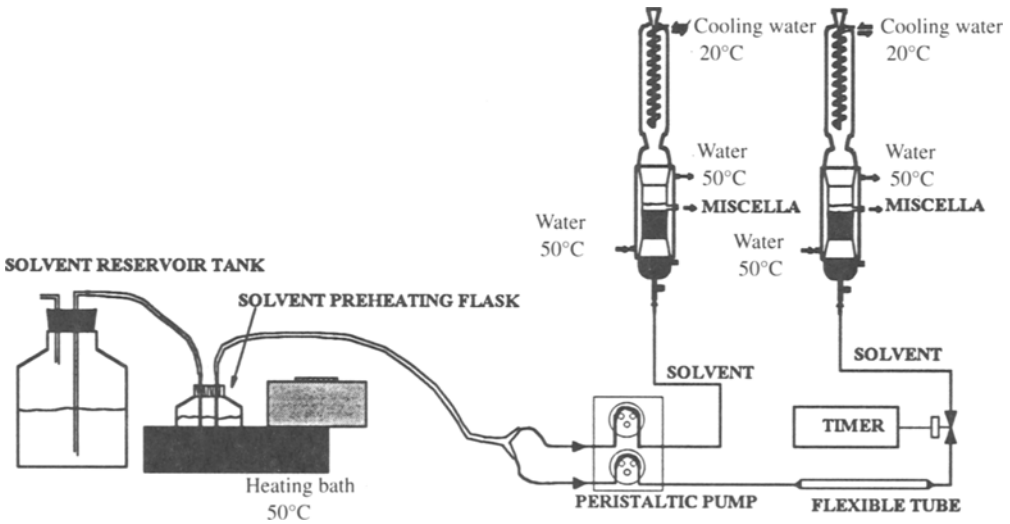

FIG. 2. Experimental pulsed extractor set-up.

TABLE 2
Results from the Repeated Batch Extraction of Polyphenols

Stage	Polyphenol concentration (mg/L)	Polyphenol extracted (mg)
1	466.20	23.31
2	188.63	9.43
3	90.95	4.55
4	79.83	3.99
5	42.27	2.11
Total		43.39

Extractable polyphenols: (g/g inert solid): 0.0255

higher concentration values were obtained in the pulsed extractor. A significance level of 0.01 (Fisher F-test) was reached by comparing the maximum deviation found in the experimental determination of polyphenol concentration and the difference between pulsed and nonpulsed extractors.

The extractable polyphenol fraction left unextracted in the meal (Y) was calculated from Equation 2, in which the extracted amount of polyphenols in the meal is expressed as a function of C_{Et}:

$$M_o \cdot C_o(1-Y) = \int_0^t Q_{Et} \cdot C_{Et} \cdot dt \qquad [2]$$

where M_o = inert solid in the bed estimated from the data shown in Table 1 (12.2 g), C_o = initial polyphenol content expressed as chlorogenic acid (0.0255 g/g inert solid), Y = fraction of extractable polyphenols left unextracted at time t, Q_{Et} = ethanol flow (2.88 mL/min), and t = extraction time (min).

The integral of Equation 2 was numerically calculated by the Romberg method (13) by using Equations 3 and 4, obtained by nonlinear regression analysis, allowing a good estimate of the concentration in the miscella with small standard error. For the pulsed system:

$$C_{Et}(\text{mg/L}) = -173.79 + 4.104 \cdot 10^{-3} \cdot t + \frac{1326.3}{\ln(t)} + \frac{7.4836 \cdot 10^3 \cdot \ln(t)}{t^2} - \frac{2.2632 \cdot 10^6}{t^2} \qquad [3]$$

$r^2 = 0.9980$; F-value = 1504; $P < 0.0001$.

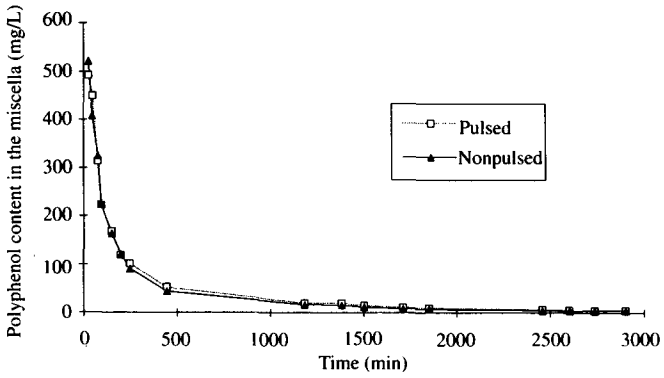

FIG. 3. Polyphenol concentration of outlet miscella during extraction in pulsed and nonpulsed extractors.

For the nonpulsed system:

$$C_{Et}(\text{mg/L}) = 3.1973 + \frac{649.71}{\left(1 + \left[\frac{t}{68.069}\right]^{1.4351}\right)} \qquad [4]$$

$r^2 = 0.9964$; F-value = 1218; $P < 0.0001$.

It is known that the extraction mechanism depends to a large degree on internal diffusion. Nevertheless, solute diffusion in vegetable materials is mainly affected by structural considerations and constraints, geometry, limited solubility, and solute interactions, and leads to behavior that deviates from that predicted by solution of the differential equations for diffusion in solids. The theory of diffusion extraction with constant diffusivity does not agree with the experimental results for pretreated (pressed or rolled) materials, where diffusion takes place in a mixed system of ruptured and unruptured cells (14). Two alternative interpretations were tried: a three-parameter model with two zones of different diffusivity (15) and a model in which diffusivity varied exponentially with the solute concentration (16).

Extraction from undamaged cells is the slowest step in the process. Some reports have shown that the extraction of polyphenols from food materials is controlled by internal diffusion (10). It can be assumed that the process for an arbitrary meal laminate in the extractor can be described by the equation:

$$D_{eff} \cdot \frac{\partial^2 C}{\partial x^2} = \frac{\partial C}{\partial t} \qquad [5]$$

where D_{eff} = effective diffusivity (m^2/s), and C = polyphenol concentration in the laminate (g/g inert solid). To resolve Equation 5, the following boundary conditions were employed: $C = 0$, $x = \pm l$, $t \geq 0$; $C = C_0$, $-l < x < l$, $t = 0$, with $2l$ being the laminate thickness. If $Y = C/C_0$, the solution of Equation 5 is given by:

$$Y = \frac{8}{\pi^2} \cdot \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \cdot e^{-\left(\frac{(2n+1)^2 \cdot \pi^2 \cdot D_{eff} \cdot t}{4l^2}\right)} \qquad [6]$$

It is generally assumed that, for Fourier number ($\pi^2 \cdot D_{eff}/[4 \cdot l^2]$) greater than 0.1 (17–19), only the first term of the series becomes significant. When this condition is attained, the effective diffusivity can be calculated by plotting ln (Y) against time (t). A linear behavior must be observed, according to:

$$\ln(Y) = \ln\left(\frac{8}{\pi^2}\right) - \frac{\pi^2 \cdot D_{eff}}{4l^2} \cdot t \qquad [7]$$

In Figure 4, the residual polyphenol content vs. extraction time has been plotted. In the pulsed extractor, the polyphenol fraction can be reduced to less than 11.8% of the initial value (corresponding to less than 0.3 g/100 g cake) after 2400 min, whereas in the immersion extractor, this separation was not achieved at the end of the experiment.

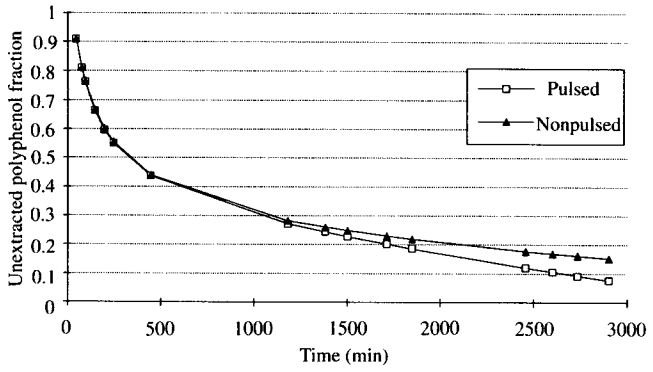

FIG. 4. Residual content of polyphenols in the meal.

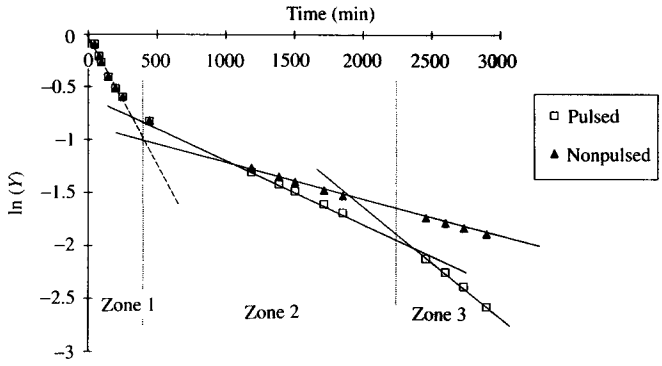

FIG. 5. Plot of linearized data according to Equation 7.

In Figure 5, a plot of ln (Y) against time is shown. It shows that the plot is not linear up to 485 min, probably because the model cannot be reduced to only one term. This fact is reflected in the low regression coefficients shown in Table 3, where calculated diffusivities are summarized. In this zone, there is no difference between pulsed and nonpulsed extractors, and both show the same curve. An average diffusion coefficient can be calculated, but it is not a reliable value. Despite this, we can compare the values for the pulsed and nonpulsed extractors, and the difference is not significant. Although nonlinear regression of the experimental data was performed according to Equation 6, taking into account five terms of the series, it did not offer a good prediction of the experimental values, confirming that it is not possible to employ only one diffusion coefficient in this zone.

After extraction for 485 min, linear behavior is observed for both pulsed and nonpulsed extractors, the difference is that two distinct zones with clearly different slopes can be observed for the pulsed extractor: one until the polyphenol content is about 15% of the initial, and the other below 15%. The second zone is not observed in the nonpulsed extractor, where only a reduction down to 15% can be reached. The extraction of polyphenols between 60 and 15% is more rapid in the pulsed extractor.

In the time range for which Equation 6 can be reduced to only one term, the diffusivity calculated for the pulsed extractor provides a minimum Fourier number value of 0.03 for 485 min. For this Fourier number, only the four first terms in the series are significant (fifth term is $1.02 \cdot 10^{-8}$). Reducing the series to only one term, instead of employing four terms, provides a 1.11% error. At this time, the Fourier module for the nonpulsed extractor is 0.018, with the error caused by reducing the five first terms in the series to only the first term being 2.66%. These errors, and those derived from the regression of experimental data (Eqs. 3 and 4) are the maximum errors caused by mathematical manipulation of the data.

The diffusion mechanism is further complicated by the phenomenon of fluid flow through a packed bed in the extractor. Wiese and Snyder (20) found that the oil was extracted much more rapidly when the solvent was forced to flow through the flakes than when flakes were suspended: about 70% of the oil extracted easily in the packed extractor, while about 30% extracted easily in the nonpacked one. In the present work, the packing degree could increase with time as a re-

Table 3
Regression Analysis and Effective Diffusivities Calculated from Data in Figure 4

	Equation 6		Effective diffusivity (m^2 /s)	
	Pulsed	Nonpulsed	Pulsed	Nonpulsed
Zone 1	$Y = 0.9979 \cdot e^{\left(-\frac{t}{387.6}\right)}$	$Y = 0.9950 \cdot e^{\left(-\frac{t}{396.9}\right)}$		
	$r^2 = 0.9788$	$r^2 = 0.9766$	$1.568 \cdot 10^{-12}$	$1.531 \cdot 10^{-12}$
Zone 2	$Y = 0.57397 \cdot e^{\left(-\frac{t}{1619.3}\right)}$	$Y = 0.4276 \cdot e^{\left(-\frac{t}{2749.2}\right)}$		
	$r^2 = 0.9990$	$r^2 = 0.9988$	$3.75 \cdot 10^{-13}$	$2.21 \cdot 10^{-13}$
Zone 3	$Y = 1.4467 \cdot e^{\left(-\frac{t}{992.9}\right)}$			
	$r^2 = 0.9998$		$6.126 \cdot 10^{-13}$	$2.21 \cdot 10^{-13}$

sult of pulsation, which could explain a greater diffusivity at zone 3. Nevertheless, Wiese and Snyder obtained behavior similar to ours in a "nonpacked bed."

The effective diffusivity values for polyphenols, found by Dibert *et al.* (10) on green coffee extraction, are between 16 and 27 times higher (as compared to pulsed and nonpulsed extractors), but the material they employed was finely ground.

For the diffusivity values obtained, a Sherwood number over 400 was obtained by using different empirical correlations (21,22). For Sherwood numbers over 200, external resistance to mass transfer can be neglected without error, diffusion being the rate-determining step, as was considered in this work.

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