EXTENDED ABSTRACT

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An environment friendly extraction process of polyphenols from apple pomaces using response surface methodology

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Abstract

The aim of this study was evaluate the potential use of low-cost and environment friendly solvents to extract polyphenols from apple wastes for biotechnological application. Apples solid wastes from industrial transformation were used as raw material to produce extracts enriched in antioxidant polyphenols, using a green and environmental friendly solvent in a solid-liquid extraction. The reaction parameters studied were the temperature (50 and70 ºC), extraction time (20 and 40 minutes), solvent ratio (ethanol:water, 20:80, 40:60, 60:40, 80:20) and solid:liquid ratio (2:1 and 4:1). Response surface methodology based on central composite design was used to assess the main impact of the different extraction factors. The phytochemical composition was assessed by HPLC-DAD/VIS-UV system and the free radical scavenging activity was measured by cupric reducing antioxidant capacity (CUPRAC) method. Our findings are important since polyphenols have an outstanding role in health area, and wide applications in food and pharmaceutical products. The optimized extraction method used in the current study might be less expensive, simple and accurate for the recovery of polyphenols based in an environmentally-friendly extraction solvent. These findings may contribute to its industrial applications for nutraceutical products development.

List of abbreviations: *Industrial transformation; food wastes; phytochemical recovery; green solvents; sustainability; added-value coproducts.*
Short title: *Recovery of polyphenols from apple pomaces*

Introduction

Every year, several million tons of apple wastes, known as “apple pomace(s)” (apple leftovers after the production of juice) are currently generated from agroindustry as results of industrial transformation of apples into juice (Kosseva, 2013). This material can be used for different purposes including pectin and citric acid extraction, enzymes production (Zheng and Shetty, 2000) as well as for polyphenol extraction (Hernandez-Carranza *et al*., 2016). In fact, apples are widely recognized as an important source of flavonols, flavanols and anthocyanidins among other polyphenols (Cao *et al*., 2009). Although the recent advances in the phytochemical extraction techniques, less is known about the best method to extract polyphenols from apple pomaces. Moreover, the majority of the solvents used in the extraction of such phytochemicals, like hexane, acetone, methanol, and ethyl-acetate (Cao *et al*., 2009; Hernandez-Carranza *et al*., 2016) are highly toxic and expensive. Therefore, with this study we aim to find a feasible alternative for polyphenol extraction from red apple pomace using environmental friendly and safe solvents (from consumers and factory workers point of

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view). In addition we aim to develop a model to maximize the polyphenol extraction and with that to establish the optimal conditions for maximization of polyphenol extraction yield.

Materials and methods

Chemicals and reagents

Ethanol, Trolox, $CuCl_2$, neocuproine and NH4Ac were purchased from purchased from Sigma-Aldrich (Teuferkichen Germany). (+)-Catechin, (-)-epicatechin, chlorogenic acid, prontocyanidin B1 and B2, coumaroyl-quinic acid, quercetin-3-O-rutinoside, quercetin-3-O-rhamnoside and cyanidin-3-O--galactoside, were purchased from Extrasynthese (Genay, France). The solvents used in sample preparation for LC-MS studies were HPLC grade.

Plant waste materials

1 Kg of red apple pomace (*Malus domestica* var. Starking) was collected from industry and freeze-dried under vacuum (Ultra-DrySystemTM), and a sub-sample of 100 g dry weight was used in a response surface model (RSM) experiment (Table 1). The extraction was performed in a block heater (Bibby Scientific Lda., UK) with agitation each five minutes in accordance with the RSM conditions. The extracts were then centrifuged (4000 rpm, 20 minutes), filtered (PTFE 0.2 µm, Ø 13 mm (Teknokroma, Spain) to amber vials and stored at 20ºC until analytical procedure.

Table 1. Independent variables and their coded and actual values used for optimization

Independent variables	Values coded	
	-1	+1
	(Actual values)	
Ethanol concentration (%), X_1	40	60
Solid: solvent ratio (mg/mL), X_2	2:1	4:1
Temperature (ºC), X_3	50	70
Time (minutes), X_4	20	40

The dependent variables were polyphenol extraction yield (**PEY**) (%) **(y1)** and total antioxidant activity (**TAA**) (µM Trolox equivalent.mL^{-1}) **(y2)**. The total polyphenol were determined by colorimetric assay in a 96-well microplates (Dewanto *et al*., 2005) and the PEY was assed trough the following formula: PEY(%) = (C x V_2) / (M x V_1 x 1000) x 100, where: **C** represents the polyphenol content (mg.mL^{-1}); $\mathbf{V_2}$, the total volume of the extracted sample (mL); **M**, the quantity of raw material (g), and $\mathbf{V_1}$, the volume tested (mL). The total antioxidant activity (TAA) was determined using the colorimetric bioassay of CUPRAC in a 96-well microplate (Sratil *et al*., 2006) and the results were expressed as µM Trolox equivalent.mL^{-1}. The data were fitted to a polynomial regression model, expressed by the following equation:

$$Y=\beta_0+\sum_{i=1}^{4}\beta_i x_i+\sum_{i=1}^{4}\beta_{ii} x_i^2+\sum_{i=1}^{3}\sum_{i=1+1}^{4}\beta_{ij}x_{ij}+\ldots$$

The best results were submitted to HPLC-DAD/VIS-UV analysis (Aires *et al*., 2013) in order to assess both polyphenol profile and content. All determinations were performed in triplicate and the data submitted to statistical analysis using the Design-Expert v.10 (20121 East Hennepin Ave., Minneapolis, USA) and SPSS v.17 (SPSS-IBM, Orchard Road-Armonk, New York, USA) software.

The imbalance between the generation of ROS and the ability of antioxidant defence systems to scavenge or degrade ROS, is termed oxidative stress, a known risk and contributing factor to various pathophysiologies including inflammation, tissue injury, carcinogenesis and neurodegeneration. Maintaining an overall redox balance is crucial for many regulatory circuits in cell signalling and tissue homeostasis.

Results and discussion

The results obtained with RSM are shown in Table 2 the model was statistically significant and the lack of fit was not significant ($p>0.05$). In addition, the values of R^2=0.985 ($p<0.001$) indicated a good fit of the models responses. Examples of surface responses are present in Figure 1 and they showed a convex characteristic, and it was possible to observe a maximum value for PEY in the combination of 50% solid:solvent ratio with 60ºC of temperature and 30 minutes of extraction time. In these conditions, the PEY and TAA was 41.94% and 1467.68 µM Trolox equivalent.mL^{-1}, respectively, whereas the initial values predicted in the model were

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41.44% and 1450.19 µM Trolox equivalent.mL^{-1}. Comparing both predicted and estimated values, they were within the range and did not vary significantly at 5% level. Thus, the regression equations obtained in this model (see below) can be used to draw extracts with higher content in polyphenols and TAA. The high regression coefficient A, B, C, D in the equation indicates that all parameters have a significant effect on the extraction of antioxidant polyphenols from apple pomace

Table 2. ANOVA results on PEY and TAA using a response surface model.

Source	(*Y*1) PEY		(*Y*2) TAA	
	F-value	*P*-value	*F*-value	P-value
Model	74.8	<0.0001	87.2	<0.0001
A-Ethanol	4.2	0.0595	4.8	0.0444
B-Solid:solvent ratio	146.2	<0.0001	181.4	<0.0001
C-Temperature	37.4	<0.0001	43.3	<0.0001
D-Time	34.7	<0.0001	40.2	<0.0001
AB	0.5	0.4738	0.6	0.4414
AC	20.8	0.0004	24.0	<0.0001
AD	0.1	0.8225	0.1	0.8092
BC	1.7	0.2072	2.0	0.1765
BD	9.6	0.0073	11.1	0.0045
CD	0.5	0.4980	0.6	0.4664
A^2	243.9	<0.0001	286.6	<0.0001
B^2	251.5	<0.0001	270.9	<0.0001
C^2	403.9	<0.0001	473.1	<0.0001
D^2	219.5	<0.0001	258.1	<0.0001
Lack of Fit	2.9	0.1238	2.5	0.1664
R^2	0.9859		0.9879	
CV	0.0250		0.0233	

The equations of regression found were:

Y1= + 41.44 - 0.34 A + 2.04B + 1.03C + 0.995D - 0.15AB + 0.943 AC + 0.047AD - 0.273BC + 0.64BD + 0.14 CD - 2.47 A2 - 2.51B2 - 3.18C2 - 2.342D2

Y2= +1450.2 - 12.05A + 73.995B + 36.2C + 34.83D - 5.32AB + 32.99AC + 1.65AD - 9.55BC + 22.45BD + 5.03CD - 87.01A2 - 84.59B2 -111.79C2 - 82.57D2

It was also found a strong correlation between TAA with PEY (R^2=0.9785, *p<0.001*) (Figure 1).

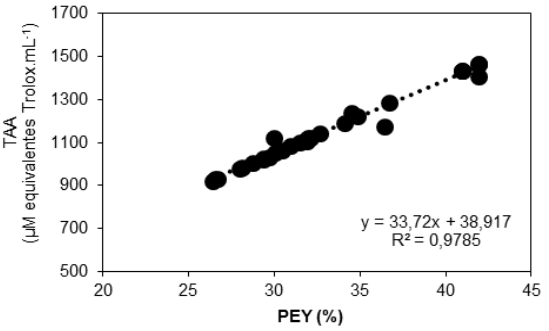

Figure 1. Relation between polyphenols extraction yield (PEY) expressed as percentage and Total antioxidant activity expressed as µM Trolox equivalent.mL^{-1} (TAA).

The main polyphenols found by HPLC-DAD analysis were: (+)-catechin (10.0±0.1 mg/100 g^{-1} dw), (-)-epicatechin (11.5±0.0, mg/100 g^{-1} dw) chlorogenic acid (15.3±0.2 mg/100 g^{-1} dw) prontocyanidin B_1 (10.2±0.1 mg/100 g^{-1} dw) and B_2(7.1±0.1 mg/100 g^{-1} dw), coumaroyl-quinic acid (20±0.3 mg/100 g^{-1} dw) quercetin-3-O-rutinoside (4.8±0.1 mg/100 g^{-1} dw), quercetin-3-O-rhamnoside (9.6.0±0.1 mg/100 g^{-1} dw) and cyanidin-3-O-galactoside (8.1 ±0.2 mg/100 g^{-1} dw9, which is in agreement with recent study of Hernández-Carranza *et al*. (2016).

To conclude the selected RSM model showed to be effective in the maximization of polyphenols extracted from red apple pomaces. Our method offered a good reproducibility, and our results can be used on a commercial scale for the extraction of polyphenols from apple byproducts for nutraceutical, pharmaceutical and/other uses.

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