Acid hydrolysis of almond shells in a biphasic reaction system: Obtaining of purified hemicellulosic monosaccharides in a single step

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HIGHLIGHTS

- Lignin-PVA smart hydrogels were successfully synthesized.
- Medium and high molecular weight PVA enabled a good synthesis.
- Lignin-PVA hydrogels presented high swelling capacities and reusability.
- The smart hydrogels were pH and temperature responsive.
- Lignin enhanced the adsorption of methylene blue cationic dye.

GRAPHICAL ABSTRACT
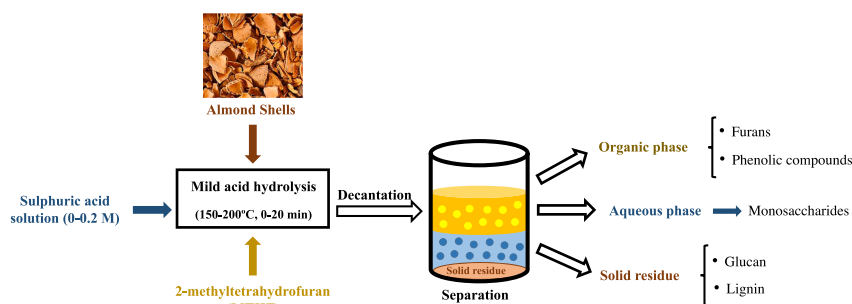
ABSTRACT

The aim of this work is to comprehend the biphasic reaction systems through another perspective; the simultaneous purification and production of carbohydrates during the pretreatment of biomass. A dilute acid hydrolysis of almond shells in a 2-Methyltetrahydrofuran/H_2O system was optimised to maximise the obtaining of hemicellulose-derived monosaccharides with the minimum formation of degradation products. The optimised conditions of the biphasic reaction system, which produced 205.3 g hemicellulose-derived monosaccharides/Kg almond shells, were replicated in a monophasic reaction system to assess the benefits of the biphasic reaction systems. The latest system allowed the removal of 85.3% of the furans generated during the dilute acid hydrolysis, creating antioxidant extract, together with the catalysis of the hydrolysis of the hemicelluloses in a 20%. Therefore, the proposed process could become a promising method to purify carbohydrates with an environmentally friendly procedure that allowed the obtaining of multiple added-value products in a single step.

1. Introduction

Hemicelluloses are one of the main components of the lignocellulosic biomass as they constitute between a 20 and 40% of feedstock, depending on the species (Kang et al., 2014). The exploitation of this fraction could permit the production of hemicellulose-derived oligo- and monosaccharides with a wide range of applications, whose constitution will depend on the employed feedstock and on the isolation procedure (Gírio et al., 2010). The oligosaccharides obtained from this fraction, due to their prebiotic and antioxidant properties have applications in the pharmaceutical and cosmetic industry, among others (Chemin et al., 2015), while the monosaccharides, mainly xylose in lignocellulosic

biomass, have been employed for the obtaining of bioethanol (Rastogi and Shrivastava, 2017), lactic acid (Bustos et al., 2004), furfural and levulinic acid (Li et al. 2019).

Between the different processes that could be employed to solubilise the hemicellulosic fraction as monosaccharides the diluted acid pre-treatment presents high effectivity (Woiciechowski et al., 2020). Nevertheless, during this treatment also part of the lignin of the feed-stock could be solubilised (Maurya et al., 2015) and degradation products such as furfural and 5-hydroxymethylfurfural (HMF) are formed (Ariffin et al., 2020). The presence of these products in the hydrolysates makes it necessary to detoxification them if they are going to be sub-jected to a fermentation process (Woiciechowski et al., 2020), since they could inhibit the growth of microorganisms precluding the fermentation or reducing its rate (McMillan, 1994). The detoxification or purification of the hydrolysates could be achieved by using activated carbon, adsorption resins, alkali compounds and enzymes (Woiciechowski et al., 2020) or by carrying out a liquid–liquid extraction with an organic solvent. The liquid–liquid extraction presents some benefits compared with the other purification methods as it permits the removal of the toxic compounds without carbohydrates loss and it allows the recycling of the solvent, being possible to carry out an integrated process (Roque et al., 2019). Furthermore, it has been reported that the extracts obtained from hydrolysates of dilute acids or autohydrolysis treatments present anti-oxidant properties due to their phenolic, furfural and HMF content (Hajji et al., 2018; Gullón et al., 2018). Taking all of this into account the aim of this work was to carry out a dilute acid hydrolysis of almond shells using a biphasic reaction system, in order to carry out the hydrolysis of the hemicelluloses and the purification of the hydrolysates in a single step. Thus, this process will permit the obtaining of purified mono-saccharides and antioxidant extract at the same time from a byproduct as the almond shells that has no industrial/commercial application but a high availability, since between 70 and 150 million tons are generated annually in Spain (de Hoyos-Martínez et al., 2018).

In the latest years, this kind of reaction systems have received a great attention since they permitted the extraction of organic compounds by the co-solvent once they are formed in the reactive phase (Saha and Abu-Omar, 2014). However, most of the examples in which biphasic reaction systems have been employed are focused on the obtaining of furans from the degradation of the lignocellulosic carbohydrates and not on the purification of the carbohydrates contained in the hyrolysates. Gürbüz et al. (2012) for instance, obtained furfural and levulinic acid by hydrolysing the hemicellulosic fraction of the corn stover using a biphasic reaction system with alkylphenol solvents. Similarly, Lucas-Torres et al. (2016) studied the obtaining of HMF and levulinic acid from the cellulosic fraction of the melon rind waste by a microwave-assisted acid catalysis carried out in a monophasic and biphasic systems. During this study, they observed that when the biphasic system composed of THF and water was employed the recovery of HMF and levulinic acid was higher than when the reaction was carried out only with water.

In this context, this work addresses the optimisation of a dilute acid hydrolysis carried out in a biphasic system composed by 2-methyltetra-hydrofuran (2-MTHF) and water, to maximise the production of hemicellulose-derived monosaccharides and minimise the formation of degradation products (HMF and furfural). 2-MTHF was chosen as organic solvent due to its low boiling point (80°C) and renewable origin, since it can be obtained from renewable resources such as furfural and levulinic acid. The 2-MTHF also presents a great potential for applica-tion in biphasic reaction systems due to its low miscibility in water and its ability to conserve the furans against the acidic hydrolytic action of water (Xue et al., 2018). Once the conditions that allowed the obtaining of the maximum amount of hemicellulose-derived monosaccharides with the minimum content of degradation products were determined, the experiment was performed using a monophasic reaction system, which was subsequently purified by a liquid–liquid extraction with 2-MTHF in order to assess the benefits of carrying out both processes simultaneously. To appreciate the advantages of carrying out the dilute acid hydrolysis of almond shells in a biphasic reaction system, the three streams obtained at optimised conditions carried out in a mono- and biphasic reaction systems were deeply characterised. The mono-saccharide and degradation products in the liquid phase were quantified by HPLC. The solid residue was chemically analysed and characterised by FTIR and pyrolysis, while the extracts obtained from the organic phase were subjected to phenolic content and antioxidant analyses.

2. Material and methods

2.1. Raw material

Almond shells of the variety Marcona were supplied by local farmers. This feedstock was milled and sieved up to 4 mm to have a single and homogeneous lot, which was kept in a dark and dry place until further use. In order to characterise them chemically, the almond shells were milled and sieved up to 0.5 mm. Their moisture, ash and extractive content were determined by standard methods (TAPPI T264-om-88, TAPPI T244-om-93 and TAPPI T204-cm-97, respectively) developed by the Technical Association of Pulp and Paper Industry (TAPPI). Conversely, the lignin, glucan and hemicellulose content of the feed-stock were analysed by a quantitative acid hydrolysis (QAH) following the TP-510–42618 protocol conceived by the National Renewable En-ergy Laboratory (NREL). The glucan and hemicelluloses hydrolysed during the QAH were quantified by HPLC using the process described by Gullón et al. (2018).

The almond shells (AS) were composed by 2.31 ± 0.03 wt% of ashes, 3.18 ± 0.12 wt% of extractives, 30.34 ± 0.81 wt% of Klason lignin, 17.41 ± 0.14 wt% of glucan and 33.52 ± 0.05 wt% of hemicelluloses (expressed as percentage of dry AS). The hemicellulosic content was measured as the joint contribution of 26.66 ± 0.18 wt% of xylan, 2.32 ± 0.12 wt% of arabinosyl substituents and 4.55 ± 0.01 wt% of acetyl groups (expressed as percentage of dry AS). The constitution of the hemicelluloses present in the almond shells, in a molar ratio, was xylose:arabinose:acetyl groups = 10:0.9:5.2. The composition of the almond shells was similar to the one determined by de Hoyos-Martinez et al. (2018).

2.2. Dilute acid hydrolysis of the almond shells in a biphasic reaction system

The dilute acid hydrolysis was performed in a 100 mL stainless steel 5500 Parr reactor by mixing a water:2-MTHF solution, with a volume ratio of 1:2 (v/v), and almond shells in a liquid/solid ratio (LSR) of 8 g/g (oven-dried basis). The water solution employed in the experiments was acidified with sulphuric acid in a range between 0 and 0.2 M. The ex-periments were performed at a temperature between 150 and 200°C, which was controlled by a 4848 Reactor controller, and using reaction times between 0 and 20 min. Once the reaction was finished, the reactor was cooled down and the liquid and solid phases were separated by filtration. While the solid phase was not used, the liquid phase was let to decant in order to separate the immiscible phases. The organic phase was vacuum evaporated at 50°C to eliminate the solvent and the ob-tained extract was stored for further analysis. On the other hand, the aqueous phase (also denoted as liquor) was analysed by HPLC following the method described by Gullón et al. (2018).

2.2.1. Experimental design and statistical analysis

The dilute acid hydrolysis was optimised by a surface response methodology (RSM) using a Box-Benken Design in order to maximise the production of hemicellulose-derived monosaccharides, which are xylose and arabinose in the case of the almond shells, and to minimise the content of the degradation products in the obtained liquor. In this design three independent variables at 3 levels (-1, 0, 1) were chosen to analyse their influence on two response variables, which were the concentration of hemicellulose-derived monosaccharides (g/L, Y_1) and the concen-tration of degradation products (g/L, Y_2). The natural values of the

independent variables together with the experimental data obtained during the 15 experimental runs are collected in Table 1. The obtained results were fed to the Statgraphic Centurion version XVI software (Statpoint Technologies Inc., Warrenton, VA, USA) and they were fitted using the following polynomial quadratic regression equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 \sum_{i=1}^{3} \sum_{j=i+1}^{2} \beta_{ij} x_i x_j \qquad (1)$$

where Y is the predicted response, β_0 is the constant coefficient, β_i, β_{ii} and β_{ij} are the regression coefficients for linear, quadratic and interaction terms and xi and xj are the independent variables. This equation allows the analysis of the influence of the independent variables on the response variables as it represents the effect of the three independent variables and their interactions concerning each of the response variables.

The commercial software Statgraphic Centurion (version XVI) was used to analyse the results and to optimise the conditions that permitted the obtaining of the maximum amount of the hemicellulose-derived monosaccharides with the minimum content of degradation products (furfural and HMF) in the liquors. The effectiveness of the models generated by the commercial software for each response variable was assessed by determining the coefficient of determination (R^2) and the adjusted R^2 coefficient by performing an analysis of variance (ANOVA). The statistical significance of each model was analysed by the *F*-test, while the significant levels of the independent variables of the model were determined for a probability level of 5% ($\alpha = 0.05$).

In order to validate the model the experiment using the optimum conditions was performed in triplicate and the obtained results were compared with the predicted values.

2.3. Dilute acid hydrolysis of the almond shells in a monophasic reaction system

The dilute acid hydrolysis of the almond shells was carried out in a monophasic reaction system using the optimum conditions determined during the experimental design, in order to study the differences between performing the experiment in a mono- or biphasic reaction system.

To perform the hydrolysis in a monophasic reaction system, the almond shells were mixed with water in a 100 mL stainless steel 5500 Parr reactor with a 4848 Reactor controller using a LSR of 8 g/g (oven-dried basis). The experiment was carried using the optimum

temperature, reaction-time and sulphuric acid concentration and once the reaction was finished the reactor was cooled down and the liquid and solid phases were separated by filtration. The solid phase was washed with water until neutral pH, air-dried and stored for further analysis. An aliquot was taken from the liquid phase for further analysis, prior to subjecting it to a liquid–liquid extraction with 2-MTHF. The liquid phase was extracted at room temperature under stirring for 15 min in a single extraction stage using a liquor:2-MTHF solution with volume ratio of 1:2 (v/v). The extraction process was carried out twice. The immiscible phases were separated by decantation and the organic phase was vacuum evaporated at 50°C to obtain organic extracts which were stored for further analysis. The aqueous phases together with the autohydrolysis liquor obtained before the liquid–liquid extraction were analysed by HPLC following the method described by Gullón et al. (2018), in order to study the ability of the liquid–liquid extraction to remove the degradation products present in the autohydrolysis liquor.

2.4. Characterisation of the different streams obtained during the optimised dilute acid hydrolysis in a mono- and biphasic reaction systems

The differences between carrying out the optimised dilute acid hydrolysis in a mono- or biphasic reaction systems were determined by comparing the composition of the different streams.

2.4.1. Characterisation of the aqueous phase
In addition to quantifying the monosaccharides, acetic acid and degradation products (furfural and HMF) in the aqueous phase obtained after the dilute acid hydrolysis carried out in a mono- and biphasic reaction systems at the optimum conditions as it is mention sections 2.2 and 2.3, respectively, their oligosaccharide content was determined following the method described by Gullón et al. (2018). The obtained hydrolysates were analysed by HPLC and the increase observed in the concentration of monosaccharides or acetic acid caused by the post-hydrolysis reflected the oligosaccharide concentration and their acetylation degree.

2.4.2. Characterisation of the solid phases
The solid residues obtained after the dilute acid hydrolysis carried out in a mono- and biphasic reaction systems at the optimum conditions were washed with water until neutral pH was achieved, air-dried, quantified and subjected to a moisture determination (TAPPI T264-om-88), to determine the solubilisation of the almond shells during the hydrolysis. To identify the effects of carrying out the dilute acid hydrolysis in a mono- or biphasic reaction system the chemical composition of the solids was determined by subjecting the solids to a quantitative acid hydrolysis as it was done with the feedstock in Section 2.1 and by carrying out FTIR and Py-GC/MS analyses.

2.4.2.1. Fourier transform infrared spectroscopy analysis (FTIR). The FTIR analysis of the solid residues and of the untreated almond shells was performed in a Two FT-IR spectrometer (PerkinElmer Spectrum). The spectras were recorded from 4000 to 600 cm^{-1} accumulating 8 scans in transmission mode and using a resolution of 4 cm^{-1}.

2.4.2.2. Pyrolysis-Gas Chromatography/Mass spectrometry analysis (Py-GC/MS). The solid residues together with the untreated feedstock were subjected to a Py-GC/MS analysis in order to identify the compounds that form part of their structure. The details of the employed equipment and methodology are described by Dávila et al. (2019). The identification of the products was carried out by comparing their mass spectra with the ones from National Institute of Standards Library (NIST) and with the ones reported in the literature (Nunes et al., 2010). The compounds considered were those with a peak area ratio higher than 0.4%. To determine their relative abundance, their peak areas were summed and normalized to 100%, as the peak area ratio could indicate the

Table 1
Operational conditions expressed in terms of the independent variables X_1 (Temperature, °C), X_2 (time, min) and X_3 (H_2SO_4 concentration, M) and their responses.

Exp. Order	Independent variables			Response variables	
	X_1 Temp. (°C)	X_2 Time (min)	X_3 [H_2SO_4] (M)	Hemicellulosic monosaccharide conc. (Y_1) (g/L)	Degradation product conc. (Y_2) (g/L)
1	175	0	0.2	2.50	1.59
2	200	10	0	3.20	0.51
3	150	20	0.1	19.84	1.02
4	175	10	0.1	4.30	1.49
5	175	0	0	1.10	0.03
6	200	10	0.2	0.76	2.09
7	150	10	0.2	20.31	0.36
8	150	10	0	1.01	0.02
9	200	0	0.1	2.65	1.31
10	175	20	0	1.72	0.03
11	175	10	0.1	2.86	1.74
12	200	20	0.1	1.73	1.62
13	175	20	0.2	1.37	1.62
14	175	10	0.1	3.81	1.44
15	150	0	0.1	16.45	0.02

concentration of the different pyrolytic products (Chen et al., 2015).

2.4.3. Characterisation of the organic phases

The oven-dried organic phases obtained for the optimised dilute acid hydrolysis carried out in a mono- and biphasic reaction systems were weighted in order to determine the extraction yield (g of non-volatile solids/100 g of oven-dried AS). These extracts were chemically characterised by the determination of their total phenolic content using the Folin-Ciocalteu method described by Singleton and Rossi (1965) and by measuring their antioxidant activity during the ABTS and DPPH tests, as described Gullón et al. (2017).

Furthermore, to make a more adequate comparison of the antioxidant activity of the obtained extracts towards the ABTS and DPPH radicals, their IC_{50} value was determined following the method described by Pan et al. (2006). This parameter represents the concentration of the extract needed to observe 50% of inhibition of DPPH or ABTS radicals.

All the analyses were carried out in triplicates.

2.5. Statistical analyses

The statistical analysis were carried out by the software IBM SPSS Statistics 24 in order to determine the differences between the results obtained with the mono- and biphasic reaction systems. The significant differences were assessed by a one-way analysis of variance (ANOVA) followed by Tukeýs test. The comparison between two sets of samples was carried out by the t student test and in all the cases the considered significant level was p < 0.05.

3. Results and discussion

3.1. Experimental design of the biphasic dilute acid hydrolysis of the almond shells

The high hemicellulosic content of the almond shells (33.52 wt%) make them a suitable feedstock for the obtaining of hemicellulose-derived monosaccharides, xylose and arabinose, by a dilute acid hydrolysis. To have a deeper insight of the treatment carried out in a biphasic reaction system, it was analysed the influence of some independent variables of the treatment as the temperature, the reaction-time and the acid concentration, on two response variables, which were the concentration of hemicellulose-derived monosaccharides (Y_1, g/L) and of degradation products (Y_2, g/L).

Table 1 shows the Box-Benkhen design matrix with the 15 experiments that were carried out and the results of the response variables, while Table 2 brings together the regression coefficients, the coefficient of determination (R^2), the adjusted R^2 coefficient for each model and the modeĺs statistical significance (Fisheŕs test). The high value of the R^2

Table 2
Equation coefficients of the model and statistical parameters.

Coefficient	Hemicellulosic monosaccharide conc. (Y_1) (g/L)	Degradation product conc. (Y_2) (g/L)
β_0	3.66	1.55
β_1	−6.16[a]	0.51[a]
β_2	0.24	0.17
β_3	2.23	0.63[a]
β_{12}	−1.08[a]	−0.17
β_{13}	−5.44	0.31
β_{23}	−0.44	0.01
β_{11}	5.58[a]	−0.32
β_{22}	0.09	−0.25
β_{33}	−2.92	−0.49[a]
R^2	91.27	94.20
R^2_{Adj}	75.57	83.75
F-value	5.81	8.97

[a] Significant coefficient at the 95% of confidence level.

coefficient (around 91.27% and 94.20% for the response variables Y_1 and Y_2, respectively) indicate the high efficiency of the models to describe the variability of the response variables. The considerably high value of $R^2_{Adj.}$ coefficient showed the good adjustment of the theoretical results predicted by the model with the experimental results, being this adjustment better for the concentration of degradation products (Y_2). This makes the developed model adequate to predict the experimental results obtained in the dilute acid hydrolysis carried out in a biphasic system. The obtained F-values (5.81 and 8.97 for the response variables Y_1 and Y_2, respectively) indicate that the obtained models were statistically significant. In general, these parameters suggested that the proposed models state adequately the relationship between the independent and the response variables.

3.1.1. Influence of the independent variables on the concentration of hemicellulose-derived monosaccharides (Y_1)

The concentration of the hemicellulose-derived monosaccharides obtained during the dilute acid hydrolysis is indicative of the effectiveness of the process to remove the hemicellulosic fraction of the feedstock and to obtain added-value products. The concentration of the hemicellulose-derived monosaccharides (specifically xylose and arabinose) achieved by the dilute acid hydrolysis in the biphasic system varied from 1.01 to 20.31 g/L (Experiments 8 and 7, respectively) as it could be seen in Table 1. In general, the experiments which were carried out without sulphuric acid a low hemicellulose-derived monosaccharide content was achieved, as an autohydrolysis process is being carried out. During the autohydrolysis process commonly, the obtaining of oligo- and polysaccharides is favoured (Lehto et al., 2017). However, if the autohydrolysis conditions are very severe the production of monosaccharides instead of the formation of oligosaccharides is favoured as it was observed by Gullón et al. (2018) when they carried out the autohydrolysis of chestnut shells in an isothermal regimen between 200 and 220°C. In order to avoid the use of very severe autohydrolysis conditions, which would favour the production of monosaccharides from the hemicellulosic fraction but also high energy demands, sulphuric acid was added to the reaction media to catalyse the breakage of the glycosidic bonds of the hemicelluloses, performing instead of an autohydrolysis a dilute acid hydrolysis. As it could be seen from the results shown in Table 1, the production of hemicellulose-derived monosaccharides was higher in the experiments in which a dilute acid hydrolysis was carried out, coinciding with what Lehto et al. (2017) suggested.

Taking into account the regression coefficient shown in Table 2, the linear effect of the temperature, the interaction effect of the temperature and the acid concentration, as well as the quadratic effect of the temperature were the variables that exerted a significant influence on the concentration of the hemicellulose-derived monosaccharides since the p-values were lower than 0.05. The polynomial quadratic regression equation (E1) that relates the independent variables with the concentration of hemicellulose-derived monosaccharides (Y_1) without taking into consideration the non-significant parameters would stay as follow:

$$Y_1(g/L) = 3.65 - 6.16X_1 - 5.44X_1X_3 + 5.58X_1^2 \qquad (2)$$

As it can be seen in this equation, the reaction time did not have a significant influence on the concentration of the obtained monosaccharides. The lack of significance of the reaction time was not in agreement with what Lee and Yu (2020) and Świątek et al. (2020) observed when they carried out a dilute acid hydrolysis of Acacia wood and spruce wood, beech wood or miscanthus. Lee and Yu (2020), for instance, suggested that although the reaction time used in the dilute acid hydrolysis of Acacia wood significantly influenced on the glucose yield, the sulphuric acid concentration exerted the strongest influenced followed by the temperature.

The effect of the temperature (X_1) and the acid concentration (X_3) on the hemicellulose-derived monosaccharide content of the liquor (Y_1)

when a middle point value was given to the reaction time ($X_2 = 0$) was shown in Fig. 1a. It could be appreciated that independently of the concentration of H_2SO_4 employed during the treatment the concentration of the monosaccharides decreased with the increase of the temperature. However, when the treatment was carried out at low temperatures, the higher the concentration of H_2SO_4 the higher was the monosaccharide production. Thus, the maximum concentration of hemicellulose-derived monosaccharides predicted by the model (20.15 g/L) was found when low temperatures and high concentration of sulphuric acid was used ($x_1 = 150°C$, $x_2 = 0$ min, $x_3 = 0.2$ M).

3.1.2. Influence of the independent variables on the concentration of degradation products (Y_2)

Since the hemicellulose-derived monosaccharides could be employed to obtain added-value compounds by fermentation, it is important to minimise the content of degradation products (HMF and furfural) in the reaction media. Thus, the concentration of the degradation products (Y_2) was the second response variable analysed during the dilute acid hydrolysis of the almond shells in a biphasic system. As it can be seen in Table 1, the concentration of the degradation products varied from 0.02 to 2.09 g/L, in experiments 8 or 15 and 6, respectively. At soft conditions, the degradation of the monosaccharides does not take place, however with the increase of the severity of the process the dehydration of monosaccharides is favoured, which consequently produce the formation of 5-HMF from hexoses and furfural from pentoses

(Kłosowski et al., 2020).

Considering the regression coefficient shown in Table 2, the linear effects of the temperature and the acid concentration, as well as the quadratic effect of the acid concentration were the variables that exerted a significant influence on the content of degradation products of the liquor. The polynomial quadratic regression equation (E1) that relates the independent variables with the concentration of degradation products (Y_2) without taking into consideration the non-significant parameters would stay as follow:

$$Y_2(g/L) = 1.56 + 0.51X_1 + 0.63X_3 - 0.49X_3^2 \tag{3}$$

As it occurred with the formation of the hemicellulose-derived monosaccharides, the reaction time did not seem to influence significantly the formation of degradation products. Gómez-Millán et al. (2019) also appreciated the higher influence of the temperature than the reaction time during the production of furfural from xylose using a water-MTHF system. According to Klosowski et al. (2020) the concentration of the mineral acid could be an important factor together with the temperature as they could favour the formation of degradation products if the reaction conditions are not optimised.

The influence of the temperature (X_1) and the acid concentration (X_3) on the concentration of the generated degradation products in the liquor (Y_2) when a middle point value was given to the reaction time ($X_2 = 0$) was displayed in Fig. 1b. From this Figure, it could be appreciated that either the increase of the temperature or the increase of the concentration of the acid would produce a raise in the content of degradation products in the liquor. Thus, the minimum concentration of the degradation products predicted by the model (0 g/L) could be obtained at the mildest conditions ($x_1 = 150°C$, $x_2 = 0$ min, $x_3 = 0$ M).

3.1.3. Optimisation of the biphasic dilute acid hydrolysis and validation of the model

The experimental design of the dilute acid hydrolysis in a biphasic reaction system apart from permitting the analysis of the influence of the temperature, reaction time and H_2SO_4 concentration on the production of monosaccharides and degradation products it allowed the optimisation of the process. The optimum conditions for the maximisation of the concentration of monosaccharides and the minimisation of the concentration of the degradation products in the liquors were: 150°C, 0 min and 0.197 M H_2SO_4. These conditions would enable the obtaining of 20.04 g/L of hemicellulose-derived monosaccharides, without the presence of degradation products.

To validate the suitability of the proposed model, the experiment under optimum conditions was carried out in triplicate. The obtained experimental results were close to the predicted values, since 24.0 ± 0.3 g/L of hemicellulose-derived monosaccharides and 0.22 ± 0.02 g/L of degradation products (furfural and HMF) were determined in the liquor. This confirmed the adequacy of the proposed model to predict the optimum conditions for the dilute acid hydrolysis.

3.2. Influence of the mono- and biphasic dilute acid hydrolysis on the obtaining of hemicellulose-derived monosaccharides

The aqueous phase obtained in the dilute acid hydrolysis treatment of the almond shells carried out in a biphasic reaction system at the optimum conditions contained 24.0 ± 0.3 g/L of xylose, since it was the only hemicellulose-derived monosaccharide identified in this liquor as it could be appreciated in Table 3. To appreciate the benefits of the biphasic reaction system, the treatment was carried out in a monophasic reaction system, being the composition of the obtained liquid phase also shown in Table 3.

The achieved results clearly reflected the advantages of carrying out the reaction in a biphasic system due to the significant differences observed between the composition of the liquors obtained in the dilute acid hydrolysis carried out in mono- and biphasic reaction systems. One
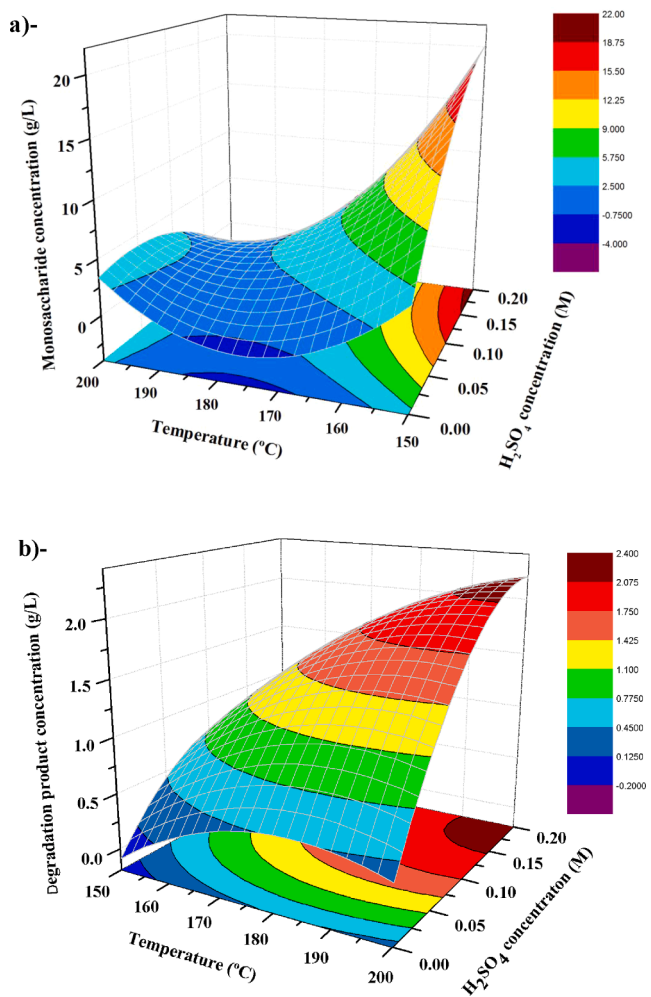

Fig. 1. Response surfaces representing the monosaccharide concentration (a) and the degradation product concentration (b) as a function of the temperature and the H2SO4 concentration at a fixed time ($x_2 = 0$).

Table 3

Composition of the aqueous fraction obtained in the dilute acid hydrolysis carried out in a biphasic system and in a monophasic system prior and after the purification with MTHF.

Composition (g/Kg AS)	Biphasic system	Monophasic system	
		Before the purification	After the purification
Glucose	3.8 ± 0.6^b	0.12 ± 0.03^a	0.14 ± 0.05^a
Xylose	205.3 ± 6.7^b	171.0 ± 10.7^a	183.8 ± 8.9^a
Arabinose	–	11.4 ± 0.9^a	12.0 ± 0.9^a
Acetic acid	14.9 ± 1.0^b	72.9 ± 2.8^c	6.8 ± 1.1^a
HMF	0.66 ± 0.04^b	0.11 ± 0.02^a	0.11 ± 0.01^a
Furfural	1.2 ± 0.2^b	12.5 ± 1.1^a	–
Glucooligosaccharides (GOS)	–	0.7 ± 0.1^a	0.79 ± 0.02^a
Xilooligosaccharides (XOS)	–	22.2 ± 2.1^a	31.2 ± 4.5^b
Arabinosyl substituents (ArOS)	–	2.9 ± 0.2^a	2.4 ± 1.5^b

In each line different letters indicate significant differences ($p \leq 0.05$).

of the benefits of carrying out the treatment in a biphasic reaction system is the lower presence of furans in the aqueous phase due to their removal from the media once they are formed. In this case, this was accomplished in the removal of furfural and surprisingly of acetic acid from the reaction media, but not in the removal of HMF (0.66 g HMF/Kg AS in the biphasic reaction system vs 0.11 g HMF/Kg AS in the monophasic one).

Regarding the carbohydrate content of the liquors obtained in the mono- and biphasic reaction systems it was appreciated that although the same reaction conditions were employed, the hydrolysis of the hemicelluloses was more effective when the treatment was carried out in the biphasic reaction system. During the dilute acid hydrolysis of the almond shells in a biphasic system 1.7 and 66.8% of the glucan and xylan present in the feedstock was converted into glucose and xylose, respectively. While, when the treatment was carried out in a monophasic reaction system, using water as the only solvent, the percentage of glucan and xylan that were converted into glucose and xylose were 0.1 and 56.4%, respectively. The lower efficiency of the hydrolysis when the treatment was carried out in the monophasic reaction system was attributed to the presence of oligosaccharides (3.55 g/L) in the obtained liquor, since around 7.3% of the xylan remained as XOS. Wang at al. (2015) also achieved a higher xylose yield when they carried out the hydrolysis of xylan using a 2-MTHF/water (5/5 v/v) system (15.8%) than when they used only water (8.2%) at the same conditions. The higher hydrolysis of the hemicelluloses when the dilute acid hydrolysis was performed in the biphasic reaction system could be explained by two different phenomena. On the one hand, the removal of the furans from the reaction media once they were formed during the hydrolysis of the hemicelluloses could thermodynamically shift the reaction towards the formation of the products favouring the hydrolysis (Esteban et al., 2020). On the other hand, the presence of MTHF could improve the hydrolytic cleave of the glycosidic bonds of the cellulose but also of the hemicelluloses. Jiang et al. (2018) explained the high conversion of cellulose in a NaCl-H_2O/MTHF system, by postulating that as the MTHF have the similar THF structure it could remove the excess of the water molecules from the hydrophobic surface of the cellulose, enhancing its cleavage by promoting the binding of the remaining water molecules to the glycosidic bonds. Since the chemical structure of the cellulose and hemicellusoses is similar, the MTHF could present the same role in the hydrolysis of the hemicelluloses.

In order to assess the selectivity of the MTHF to remove only furans from the reaction media, the liquor obtained when the treatment was carried out in the monophasic reaction system was purified by a liquid–liquid extraction using MTHF as the organic solvent. The results

shown in Table 3 reflected that the MTHF employed in the liquid–liquid extraction was able to remove furfural and surprisingly acetic acid from the liquor obtained in the monophasic reaction system (90.6% of the acetic acid in the media was removed), but not HMF. The low ability of the organic solvent to extract carbohydrates was also appreciated, since the monosaccharide content of the liquor was not affected and the oligosaccharide content of the liquor increased after the purification.

Thus, carrying out the dilute acid hydrolysis in a biphasic not only permitted the selective removal of the furans and acetic acid from the reaction media but also promoted the hydrolysis of the hemicelluloses.

3.3. Influence of the mono- and biphasic dilute acid hydrolysis on the solid residues

The solid residues obtained during the dilute acid hydrolysis carried out at the optimum conditions in the mono- and biphasic reaction systems were chemically characterised in order to analyse the influence of the reaction system. The hemicellulosic content of the solid residues obtained after each treatment permitted the determination of the efficiency of the hydrolysis of this fraction. These analyses confirmed that the hydrolysis in the biphasic system occurred to a greater extended (99% of the hemicelluloses of the untreated almond shells were eliminated in the biphasic reaction system and 74.2% in the monophasic one).

The removal of the hemicellulosic fraction independently of the employed reaction system permitted the obtaining of solids mainly constituted by glucan (18.1 wt% of the oven-dried solid residue independently of the employed reaction system) and lignin (50.3 and 56.01 wt% of the oven-dried solid residues obtained in the mono- and biphasic reaction systems, respectively). The lack of solubilisation of the lignin fraction during the dilute acid hydrolysis independently of the employed reaction system could be associated with the use of not adequate reaction conditions. Since, Schmetz et al. (2020), for instance, were able to remove 87% of the lignin present in sugar cane bagasse when they carried out a treatment using 1% (v/v) of H_2SO_4 in a H_2O/butanol system for 45 min at 180°C. Moreover, Zhang et al. (2020) achieved the removal of the 60% of the lignin content of the cooking rice straw by subjecting it to a process in which 0.5 M of H_2SO_4 in a H_2O/2-phenoxyethanol system was used for 2 h at 130°C. These authors were able to hydrolyse only 10% of the cellulose present in their feedstock, while in this case, 37.5% of the glucan of almond shell was removed in the biphasic reaction systems. This increase in the glucan removal could be associated with the employment of MTHF. Although the employment of other acid catalyst such as 2,5 furandicarboxylic acid or $AlCl_3$ could improve the hydrolysis of the cellulose (Dávila and Labidi, 2020), it is the NaCl-H_2O/THF system employed by Jiang et al. 2020, which permitted the removal of 86.8% of the cellulose present in pubescens (*Phyllostachys heterocycla cv. pubescens*) by its conversion in oligosaccharides without the employment of catalysts. In this case, the benefits of the THF in the hydrolysis of the cellulose is enhanced by the sodium hydroxide which could also activate the autocatalytic hydrolysis of the cellulose.

3.3.1. Influence of the dilute acid hydrolysis on the structure of the solid residues

Although it was observed that the dilute acid hydrolysis did not significantly the lignin content of the almond shells independently of the employed reaction system, the obtained solid residues together with the untreated almond shells were analysed by FTIR and Py-GC/MS.

Although the FTIR spectra of the three analysed solids were similar, some variations could be appreciated. These variations could be due to the effects caused by the dilute acid hydrolysis on the structure of the almond shells. The removal of the hemicellulosic fraction form the almond shells could be observed in the decrease of the intensity of the bands shown at 1740, 1600 and 1234 cm^{-1}. These bands, corresponded to the C = O stretching, to the asymmetric COO^- stretching and to the C-

O stretching of the acetyl groups bound to the hemicelluloses (Michell and Higgins, 1999; Giudicianni et al., 2018; Gullón et al., 2011). While the bands observed at 1740 and 1234 cm^{-1} disappeared in the solid residue obtained in the biphasic reaction system due to the complete removal of the hemicellulosic fraction, this did not occurred with the band observed at 1600 cm^{-1}, as it is also associated to the vibration of the aromatic skeleton of lignin. Other two bands associated with this vibration were the ones observed at 1505 and 1420 cm^{-1}, which presented a higher intensity in the obtained solid residues (Dávila et al., 2019). Furthermore, the FTIR spectra of the solid residues presented some bands at 1108 and 1060 cm^{-1} that were not appreciated in the spectra of the untreated almond shell. The first band corresponded to the in-plane deformation of the syringyl ring (Erdocia et al., 2014), while the second one corresponded to the C–C and C-O bonds stretching in the C6 of the cellulose (Galia et al., 2015). These bands were could be appreciated only in the FTIR spectra of the soild residues, since due to the removal of the hemicellulosic fraction they are mainly constituted by glucan and lignin.

Although the FTIR spectra recorded from the solid residues obtained during the dilute acid hydrolysis did not presented significant differences, their composition was significantly different as it was appreciated in the results obtained from the pyrolysis analyses. The variations observed in the distribution of the pyrolysis products obtained from each solid residue and from the untreated almond shell (Table 4), demonstrated the effect of the dilute acid hydrolysis on the structure of the lignin fraction of the solids.

Among the determined pyrolysis products, the lignin-derived compounds accounted from 42.17% in the untreated almond shells to 28.78% in the solid residue obtained in the dilute acid hydrolysis carried out in the monophasic reaction system. A similar amount of lignin-derived products were identified during the pyrolysis of lignins isolated from walnut (50%) or chestnut shells (20%) (Chen et al., 2015; Morales et al., 2018). The lower content of lignin-derived compounds determined during the pyrolysis of the solid residues obtained during

the dilute acid hydrolysis could be associated to their more condense structure. The distribution of the type of identified lignin-derived products also suggested that the structure of the lignin fraction of the almond shells have been changed during the treatment, since the ratio of syringol and guaiacol derived compounds (S/G) was 0.69 for the untreated almond shell and 0.23 for the solid residue obtained in the biphasic reaction system.

Although the lignin content of the untreated almond shells was not altered during the dilute acid hydrolysis, it has been observed that the treatment has changed its structure. Commonly, when the dilute acid hydrolysis treatments are carried out in monophasic reaction systems, once the lignin is solubilise it is supposed to condense and relocate back on the surface of the feedstock (Patri et al., 2021). However, the addition of a co-solvent such as THF and due to their structural similarity the MTHF, would permit the solvation of the lignin fraction preventing its self-aggregation and re-deposition (Patri et al., 2021), since the solvent-lignin interaction are energetically more favoured than the lignin-lignin interactions (Smith et al., 2016). Thus, although the employment of biphasic reaction should favour the solubilisation the lignin fraction, this was not appreciated in this work independently of the employed reaction system. The lack of lignin solubilisation observed during the dilute acid hydrolysis carried out in a monophasic reaction system could be associated to repolymerisation reactions, while it could be associated with the employment of not suitable conditions in the case of the biphasic reaction system.

3.4. Influence of the mono- and biphasic dilute acid hydrolysis on the obtaining of antioxidant extracts

The performance of the dilute acid hydrolysis in a biphasic reaction system would permit the removal of the solubilised phenolic monomers and of furans generated from the reaction media, forming organic extracts. These type of extracts are receiving great attention due to their antioxidant and antimicrobial activities (Moreira et al., 2016; Zhao

Table 4
Distribution of the products identified during the Pyr-GC/MS analyses of the untreated almond shell (AS) and obtained solid residues.

Compound	Retention time (min)	Origin[a]	Main fragments (m/z)	Area % Untreated AS	Biphasic	Monophasic
Toluene	4.47		91, 92, 65	–	0.81	–
Furfural	5.05	Carb.		2.95	2.31	8.54
5-methyl furfural	7.22	Carb.	110, 109, 53	0.59	2.36	1.33
Phenol	7.55	H	94, 66, 65	1.49	2.32	–
2-hydroxy-3-methyl-2-cyclopenten-1-one	7.56	Carb.	112, 55, 69	–	–	0.77
2-methyl phenol	8.64	H	108, 107, 77	–	0.62	–
4-methyl phenol	9.07	H	107, 108, 77	0.89	3.63	0.97
Guaiacol	9.35	G	109, 124, 81	9.16	1.73	5.57
2,4-dimethyl phenol	9.69	H	107, 122, 121	–	0.57	0.71
3-ethyl-2-hydroxy-2-cyclopenten-1-one	9.89	Carb.	126, 55, 83	1.13	–	
3-methyl guaiacol	10.48	G	123, 138, 77	–	–	0.78
4-methyl guaiacol	11.36	G	138, 123, 95	3.85	12.71	8.88
catechol	11.77	C	110, 64, 63	3.93	–	–
3-methoxy catechol	13.51	C	140, 125, 97	2.16	0.65	–
4-ethyl guaiacol	13.78	G	137, 152, 122	2.43	0.80	1.97
4-methyl catechol	14.49	C	124, 123, 78	1.07	6.70	–
Syringol	16.12	S	154, 139, 111, 96	11.73	1.56	6.94
Vanillin	17.25	G	151, 152, 81	1.36	0.65	0.90
Cis-Isoeugenol	17.31	G	164, 149, 77	1.14	–	–
Acetovanillone	18.94	G	151, 166, 123	0.62	–	–
4-allyl syringol	20.74	S	194, 91, 119	1.21	0.58	0.77
Syringaldehyde	21.22	S	182, 181, 111	–	1.66	0.72
Acetosyringone	21.72	S	181, 196, 182	–	–	0.57
Palmitic acid	24.98	–	73, 60, 55	5.84	21.37	10.48
Stearic acid	26.85	–	73, 284, 60	2.26	5.83	1.45
Lignin derived compounds (%)				42.17	34.18	28.78
Total syringyl derivates (%)				12.94	3.80	9
Total guaiacyl derivates (%)				18.56	15.89	18.1
S/G ratio				0.69	0.23	0.50

[a] The identified lignin-derived compounds were classified depending on their aromatic structure, as guaiacol (G), phenol (H), syringol (S) or catechol (C) derivates (Wang et al., 2015).

et al., 2013) and are starting to be use for the obtaining of bioactive materials such as food packages (Moreira et al., 2016) or hydrogels (Huamán-Leandro et al., 2020). Thus, the comparison of the phenolic content and antioxidant activity of the organic extracts obtained during the dilute acid hydrolysis carried out in a biphasic system at the optimum conditions and after the purification of the liquor obtained in the treatment carried out with the monophasic reaction system was carried out.

The phenolic compounds extracted from the almond shells during the monophasic dilute acid hydrolysis (0.60 ± 0.08 g GAE/100 g oven-dried almond shells) were lower than the ones obtained during the biphasic reaction system (1.7 ± 0.5 g GAE/100 g oven-dried almond shells). However, they were similar to the ones obtained by Moure et al. (2007) when they carried out a solvent extraction of the almond shells acid hydrolysates, obtained at $120°C$ for 2 h with 1% of sulphuric acid (≈ 0.6 g GAE/100 g oven-dried AS). They observed that the increase of the severity of the dilute acid hydrolysis treatment would permit the higher production of monomeric phenolic compounds, however it would also consequently cause the hydrolysis of the monosaccharides disfavouring their obtaining. The higher amount of phenolic compounds extracted from the almond shells during the biphasic reaction system is associated to the quantity of organic extracts obtained during the biphasic reaction. The dilute acid hydrolysis carried out in the biphasic reaction system at the optimum conditions allowed the obtaining of 7.6 ± 0.6 g extract/100 g oven-dried almond shells, while 0.27 ± 0.07 g extract/100 g oven-dried almond shells were achieved after the purification of liquor obtained with the monophasic reaction system.

Regarding the antioxidant activity of the extracts, the ones obtained during the biphasic reaction system presented a higher ability to scavenge the ABTS and DPPH free radicals (2.9 ± 0.4 and 0.38 ± 0.06 g TE/100 g oven-dried almond shells, respectively) than the ones obtained in the monophasic reaction system (1.0 ± 0.3 and 0.017 ± 0.001 g TE/100 g oven-dried almond shells, respectively). This could be, on the one hand, because the biphasic reaction system permitted the obtaining of a higher quantity of extracts and because they presented a higher antioxidant activity as it was reflected in the IC_{50} values of the two extracts determined by the ABTS and DPPH tests. 0.65 and 0.93 mg/mL of the extract obtained in the biphasic reaction system were need to inhibit 50% of the action of the DPPH and ABTS free radicals, while 0.57 and 0.31 mg/mL of the extracts obtained in the monophasic reaction system were needed, respectively. The scavenging activity of the extracts obtained in the biphasic reaction system was higher against the ABTS free radical but lower against the DPPH free radical than the one reported by Gullón et al. (2017) for the extracts obtained from the autohydrolysis of vine shoots at $195°C$ (2.4 ± 0.1 and 0.73 ± 0.02 g TE/g oven-dried vine shoots, respectively).

4. Conclusions

The advantages of the biphasic reaction systems were assessed during the dilute acid hydrolysis of the almond shells. The optimised dilute acid hydrolysis in a biphasic reaction system was simulated using a monophasic reaction system in order to identify the advantages of these systems. It was appreciated that apart from catalysing the hydrolysis of the hemicelluloses, they permitted the removal of furfural, acetic acid and phenolic monomers obtaining organic extracts with high antioxidant activity. All these benefits would make the biphasic reaction systems a promising implementation for very diverse biorefinery treatments.

CRediT authorship contribution statement

Izaskun Dávila: Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft. **Estelle Diaz:** Validation, Investigation. **Jalel Labidi:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.125311.

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