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# *Application of a Doehlert experimental design for optimizing the dioxane organosolv extraction of lignin from hydrolyzed almond shells*

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*Aplicación de un diseño de experimentos de Doehlert para la optimización de la extracción organosolv con dioxano de cáscaras de almendra hidrolizadas*

*Aplicació d'un disseny d'experiments de Doehlert per a l'optimització de l'extracció organosolv amb dioxà de closques d'ametlla hidrolitzades*

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## **RESUMEN**

Se aplicó un diseño de experimentos de Doehlert para tres factores para el estudio de la solubilidad en mezclas acuosas de 1,4-dioxano (concentración de dioxano, 0-100% vol.) y en diferentes condiciones de extracción [temperatura de extracción (150-230°C) y tiempo de cocción (10-40 min)] de la lignina contenida en cáscaras de almendra (*Prunus amygdalus*) hidrolizadas. Las variables que más influencia tenían sobre el proceso de extracción organosolv fueron la temperatura de extracción y la concentración de dioxano; en el caso del grado de deslignificación alcanzado y de la selectividad en la extracción de la lignina, ambas variables ejercieron una influencia similar, mientras que la extractabilidad del material se vio más afectada por la temperatura de extracción. La influencia del tiempo de cocción fue pequeña en todos los casos. Además, se observó una dependencia significativa entre la temperatura de extracción y la concentración óptima de dioxano de la mezcla que conducía a un grado de deslignificación máximo, lo que fue explicado mediante la teoría del parámetro de solubilidad y teniendo en cuenta tanto la degradación de la estructura de la lignina como los cambios ocurridos en la asociación entre la lignina y los carbohidratos.

**Palabras clave:** Extracción organosolv con dioxano, Diseño de experimentos de Doehlert, Cáscaras de almendra hidrolizadas, Condiciones de extracción de la lignina.

## **SUMMARY**

The solubility of lignin from hydrolyzed almond (*Prunus amygdalus*) shells in 1,4-dioxane–water mixtures (dioxane concentration, 0-100% vol.) and different extraction conditions [extraction temperature (150-230°C) and cooking time (10-40 min)] was studied using a three-factor Doehlert experimental design. The most influenced variables in the organosolv process were the extraction temperature and the dioxane concentration; in the case of the delignification degree reached and the selectivity of the lignin extraction, both variables had a similar influence, while

extractability was most influenced by the extraction temperature. The influence of cooking time was small in all the cases. Moreover, a significant dependence was observed between the extraction temperature and the optimum dioxane concentration of the mixture leading to maximum delignification, which was explained by solubility parameter theory and taking into account changes in both the extent of the association between lignin and carbohydrates and the degradation of the lignin structure.

**Keywords:** Dioxane organosolv extraction, Doehlert experimental design, Hydrolyzed almond shells, Lignin extraction conditions.

## **RESUM**

Es va aplicar un disseny d'experiments de Doehlert per a tres factors per l'estudi de la solubilitat en mesclades aquoses de 1,4-dioxà (concentració de dioxà, 0-100% vol.) i en diferents condicions d'extracció [temperatura d'extracció (150 - 230 °C) i temps de cocció (10-40 min)] de la lignina continguda en closques d'ametlla (*Prunus amygdalus*) hidrolitzades. Les variables que més influència tenien sobre el procés d'extracció organosolv van ser la temperatura d'extracció i la concentració de dioxà, en el cas del grau de deslignificació assolit i de la selectivitat en l'extracció de la lignina, ambdues variables exerciren una influència similar, mentre que l'extractabilitat del material es va veure més afectada per la temperatura d'extracció. La influència del temps de cocció va ser petita en tots els casos. A més, es va observar una dependència significativa entre la temperatura d'extracció i la concentració òptima de dioxà de la mescla que conduïa a un grau de deslignificació màxim, el que va ser explicat mitjançant la teoria del paràmetre de solubilitat i tenint en compte tant la degradació de la estructura de la lignina com els canvis ocorreguts en l'associació entre la lignina i els carbohidrats.

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**Paraules clau:** Extracció organosolv amb dioxà, disseny d'experiments de Doehlert, closques d'ametlla hidrolitzades, condicions d'extracció de la lignina.

## 1. INTRODUCTION

The processes in which raw materials are pulped produce large amounts of wastewater of a highly polluting nature, especially sulfite- and sulfate-based process. One way of circumventing this shortcoming is by removing the fibre and lignin using suitable organic solvents (cellulose pulping processes based on sulfur-free organic solvents, *viz.* organosolv processes) [1]. Although the effectiveness of such solvents has long been known, they have only recently started to be used for this purpose at pilot-scale and in small-scale industrial plants [2], largely as a result of the scarcity of effective alternatives to conventional processes and the need to respond to new economic and environmental challenges.

Organosolv processes have been applied with varying degrees of success to hardwood and softwood (92–95% of the raw materials used to obtain paper) and also, to a lesser extent, to non-wood materials. A number of non-wood materials have been shown to provide excellent fibre for speciality paper and are the sole viable raw materials available for this purpose in some geographic areas. Also, non-wood materials, such as agricultural residues, may help alleviate the growing scarcity of forest wood materials [3].

Current organosolv pulping methods are intended to provide not only pulp, but also lignin, sugars and other by-products so as to exploit the full potential of the raw materials. In addition, organosolv pulping shows promise for use in biorefinery processes; for example, Lignol Innovations (Vancouver, Canada) has developed a biorefining technology that uses an ethanol-based organosolv step to separate lignin, hemicellulose components and extractives from the cellulosic fraction of woody biomass; the resultant cellulosic fraction is readily converted to ethanol by saccharification and fermentation, while the liquor from the organosolv step is processed to recover lignin, furfural, xylose, acetic acid and a lipophilic extractive fraction [4]. The lignin produced (a by-product of delignification) by conventional pulp and paper mills is degraded, so that it is only used in low-added value applications and energy production. Organosolv processes, on the other hand, especially those that do not use catalysts, generate a large amount of a high-quality lignin which is relatively pure, primarily unaltered and less condensed than other pretreatment lignins. These "unaltered" lignins are expected to play an important role in the near future as raw materials for the production of bio-products because they are soluble in many organic solvents and could find applications in the fields of adhesives, fibres, films and biodegradable polymers.

Organosolv delignification is favoured by the addition of catalysts (inorganic acids). However, the lignin solubilized in the presence of these acid catalysts presents a higher degree of degradation [5], hindering its further use in the field of fine chemicals; moreover, from an economic standpoint, the addition of catalysts makes the organosolv process more expensive.

Pulping processes have been subjected to factor design testing in order to develop polynomial models capable of

predicting the properties of the pulp and paper sheets. The obtained models are a function of the operating variables, which permits optimization of the responses [6-8].

In this work, we study the 1,4-dioxane organosolv extraction of lignin from a solid lignin-rich industrial waste (hydrolyzed almond shells) resulting from the production of xylose from almond (*Prunus amygdalus*) shells. To minimize lignin degradation and to reduce production costs, the extraction was carried with no added catalysts. The main variables affecting the delignification process (1,4-dioxane concentration, extraction temperature and cooking time) were investigated by applying a three-factor Doehlert experimental design, and the results were interpreted by means of the solubility parameter theory.

## 2. MATERIALS AND METHODS

### 2.1. Starting material

Hydrolyzed almond shells (HAS), a solid residue from a xylose production process (treatment with 1N H<sub>2</sub>SO<sub>4</sub> at reflux and atmospheric pressure), were supplied by Furfural Español, S.A. (Alcantarilla, Murcia, Spain). Most of the hemicelluloses were hydrolyzed in these mild conditions, with the consequent formation of xylose, leaving a solid residue, whose biopolymeric structure (cellulose and lignin) is not excessively disturbed [9]. In contrast, the hydrolysis conditions used to produce furfural are more severe (treatment with 3% H<sub>2</sub>SO<sub>4</sub> at 180°C and 10 bar for 2 h), resulting in a much more degraded residue [10], which is less suitable for an organosolv extraction study.

Before organosolv extraction, HAS were ground in a hammer mill, sieved to a particle size of 0.2–0.5 mm, extracted with water in a soxhlet to lower the acidity (to pH>5) and eliminate the remaining hydrolyzed hemicelluloses, and, finally, dried in an oven at 45°C for at least 12 h. The material consisted of the following (expressed in % weight on dry basis): lignin, 47.4; holocellulose, 48.4; cellulose, 36.3; pentosans, 5.9; ash, 0.02; and moisture, 4.5.

### 2.2. Analytical methods

The Klason lignin, holocellulose, cellulose, pentosans and ash were determined by the ASTM D1106-96, ASTM D1104-56, ASTM D1103-60, ASTM D1787 and ASTM D1102-84 norms, respectively.

### 2.3. Working method

Before organosolv extraction, the HAS were humidified to reach a 22% moisture content and stored at 20°C in darkness for at least two days. This led to the highest extractability of the material during organosolv extraction (internal communication) due to the swelling of its structure.

Next, organic suspensions (8 g of HAS in 150 ml of solvent mixture) were prepared and left for 24 h in darkness before the process was started. The suspensions were loaded in batches into a reactor (a stainless steel cylindrical autoclave of 300 ml capacity), which was then pressurized with nitrogen up to 4.9 MPa. The organosolv extraction was carried out by introducing the reactor (by means of a mechanical-pneumatic arm) into a smelted salt bath, which permitted the reaction mass to reach the desired temperature in less than 9 min; the heating time needed for the reaction mass to reach the extraction temperature was not considered as part of the cooking time. The reactor was continuously shaken orbitally at 100 rpm during extraction.

After the reaction time, the reactor was removed from the salt bath and introduced in a water bath to stop the reaction [11].

After treatment, the solid and liquid phases were separated by filtration and the solid was washed with 100 ml of the same solvent mixture as used for the organosolv extraction. The solid obtained was then dried in an oven at 105°C and weighed. The liquid phase and the wash liquid were mixed, brought to 250 ml with the solvent used for extraction and stored in 250 ml bottles in the refrigerator.

## 2.4. Experimental design

Since the classical method of varying one variable at a time has the disadvantage of not being able to consider the effect of interaction between variables, which may lead to erroneous or inaccurate results, an experimental design based on a three-factor Doehlert uniform network was applied to determine the synergic effect between variables. To apply this experimental design, ranges of 0/100-100/0 v/v (0-100% vol. of dioxane), 150-230°C and 10-55 min were selected for the dioxane-to-water ratio (dioxane concentration), extraction temperature and cooking time, respectively. This design meets the general requirements that every parameter in a polynomial model should be estimated from a reduced number of observations and that observations should be spread as evenly as possible over the experimental region of interest. With three independent variables, fifteen distinct experiments were sufficient to fit a second-order polynomial model. The central combination for the experimental design was a dioxane concentration of 50% vol. of dioxane, an extraction temperature of 190°C and a cooking time of 25 min. The experiment corresponding to the central combination was repeated twice to estimate the experimental error. The results were subjected to multiple lineal regression in a Microsoft Excel spreadsheet.

## 3. RESULTS AND DISCUSSION

The following parameters (all expressed as % dry weight) were defined to monitor the organosolv process: extractability (amount of starting material which has been dissolved), delignification (amount of Klason lignin which has been dissolved) and selectivity (amount of Klason lignin present in the dissolved matter). The delignification was determined based on the difference between the amount of Klason lignin contained in the initial material and that present in the solid residue after extraction. The Klason lignin content of the solid materials was determined twice and the error in its determination was  $\pm 3.1\%$ . The selectivity was calculated as delignification multiplied by the Klason lignin content (in % weight) of the initial material and divided by the extractability.

The application of the three-factor (study variables) Doehlert uniform network led to mathematical models capable of predicting and optimizing the extractability, the delignification and the selectivity in the experimental domain of the 1,4-dioxane concentration, extraction temperature and cooking time. The proposed experimental design was used to obtain the results given in Table 1, in which the experimental conditions used are also shown. When the central operating conditions (50% vol. of dioxane, 190°C and 25 min) were used in three experiments (essays number 1, 8 and 15), the results obtained in each

experiment for the extractability, delignification and selectivity differed by less than 6.3, 7.7 and 8.3%, respectively, from their average values. Experimental data were modeled using the second order polynomial

$$Z = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2$$

where Z represents the response studied,  $X_1$ ,  $X_2$  and  $X_3$  the coded independent variables (corresponding to the dioxane concentration, extraction temperature and cooking time, respectively), and  $a_0$ ,  $a_1$ ,  $a_2$ ,  $a_3$ ,  $a_{12}$ ,  $a_{13}$ ,  $a_{23}$ ,  $a_{11}$ ,  $a_{22}$  and  $a_{33}$  the regression coefficients, which must be estimated from the experimental data in Table 1. The expressions that relate the real value of each natural variable with its coded value are:

**Table 1.-** Experimental design conditions and results for dioxane organosolv extraction from HAS.

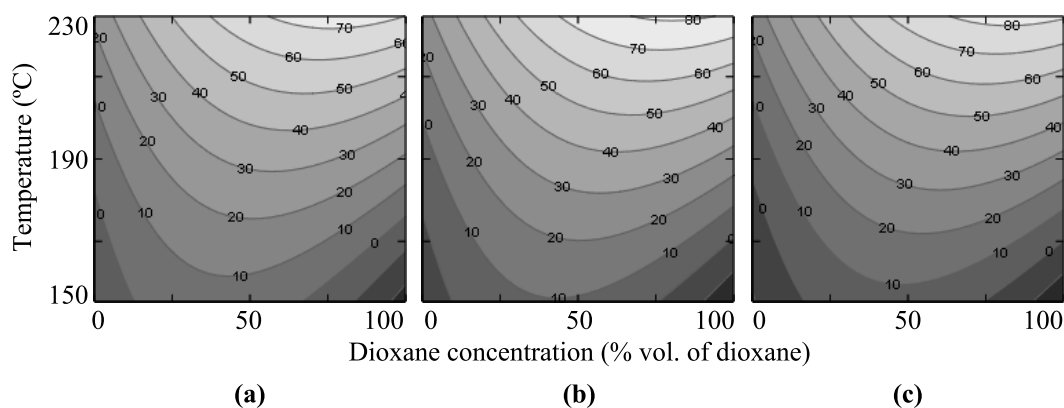
Essay N°	P (v/v)	T (°C)	t (min)	E (%)	LK (%)	D (%)	S (%)
1	50/50	190	25	39.2	32.1	58.8	71.0
2	100/0	190	25	17.4	34.9	39.2	100
3	75/25	230	25	83.9	68.2	76.9	43.4
4	25/75	230	25	51.7	85.2	13.2	12.1
5	0/100	190	25	13.6	52.8	3.7	12.9
6	25/75	150	25	4.1	45.8	7.4	85.1
7	75/25	150	25	3.8	45.4	7.8	96.7
8	50/50	190	25	37.2	37.0	51.0	65.0
9	25/75	203	40	28.6	49.6	25.3	41.9
10	50/50	163	40	15.2	40.0	28.4	88.9
11	75/25	203	40	56.8	20.8	81.0	67.6
12	25/75	177	10	10.5	45.4	14.2	64.3
13	50/50	217	10	55.4	62.5	41.2	35.3
14	75/25	177	10	22.9	31.6	48.6	100
15	50/50	190	25	34.7	31.9	56.1	76.6

$P$ = dioxane-to-water ratio;  $T$ = extraction temperature;  $t$ = cooking time;  $E$ = extractability;  $LK$ = Klason lignin in the extraction residue;  $D$ = delignification;  $S$ = selectivity.

Dioxane concentration (% vol.) =  $50 + 50 X_1$ ;  $-1 < X_1 < +1$   
 Extraction temperature (°C) =  $190 + 46.19 X_2$ ;  $-0.866 < X_2 < +0.866$ .

Cooking time (min) =  $25 + 18.38 X_3$ ;  $-0.816 < X_3 < +0.816$

The estimated regression coefficients, together with the  $r^2$  values and Snedecor's  $F$ -values for the fitted models, are shown in Table 2. In the coefficients calculated for the extractability, it can be observed that the most influential factor was the extraction temperature, followed by the dioxane concentration and finally by the cooking time ( $a_2 > a_1 > a_3$ ), the only appreciable interaction being between temperature and dioxane concentration ( $a_{12}$ ). Of the quadratic terms, only term  $a_{11}$  was significant, its negative value indicating the possible presence of a maximum extractability value within the dioxane concentrations used. As the cooking time had little influence on this response (low value of  $a_3$ ) and on delignification and selectivity, only the contour plots in the experimental domain of the dioxane concentration and extraction temperature for cooking times of 10, 25 and 40 min will be presented. As can be seen in Figure 1, both dioxane concentration and extraction temperature significantly affected the extractability, whereas the cooking time hardly influenced it. It can also



**Figure 1.-** Predicted contour levels for the extractability (wt%) of HAS as a function of dioxane concentration and extraction temperature for cooking times of: a) 10, b) 25, and c) 40 min.

be observed that the amount of starting material dissolved passed through a maximum for dioxane concentrations of 40-80% vol., depending on the extraction temperature: the higher the extraction temperature, the greater the maximum extractability obtained, and the more this maximum moves towards increasing concentrations of dioxane. In addition, regardless of the dioxane concentration, the extractability increased considerably and continuously with increasing extraction temperatures and only slightly with the cooking time. The use of the Solver tool of Microsoft Excel for optimizing the second order polynomial led to a maximum extractability value of 83.5% when using a dioxane-water mixture of 83% vol. of dioxane at the maximum temperature tested (230°C) and for a cooking time of 37.6 min.

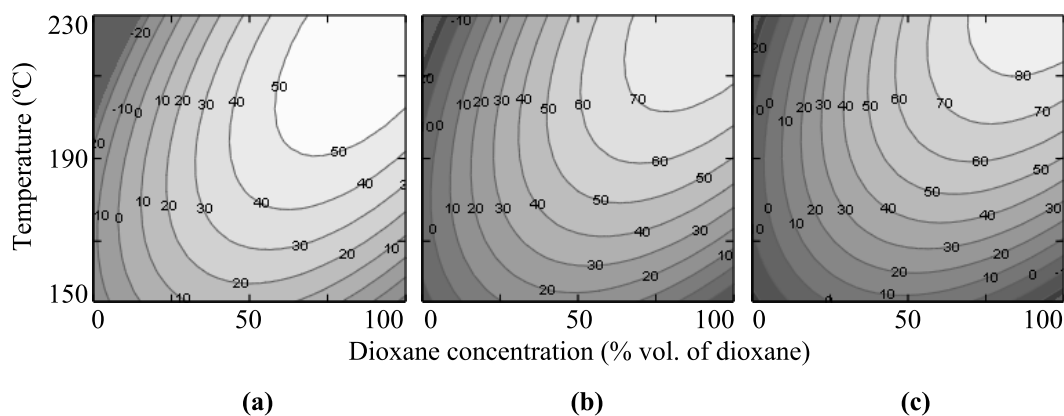
**Table 2.-** Regression coefficients of the model,  $r^2$  values and Snedecor's  $F$ -values for dioxane organosolv extraction from HAS.

	Extractability	Delignification	Selectivity
$a_0$	37.03	55.29	70.90
$a_1$	10.02	28.14	34.79
$a_2$	37.21	21.20	-39.04
$a_3$	2.39	6.26	-0.24
$a_{12}$	18.81	36.57	11.43
$a_{13}$	2.97	0.10	-10.15
$a_{23}$	1.71	12.89	10.43
$a_{11}$	-21.51	-33.83	-14.44
$a_{22}$	5.65	-27.37	-10.63
$a_{33}$	-4.25	-7.98	-0.61
$r^2$	0.961	0.938	0.960
$F$	17.68	11.05	4.22

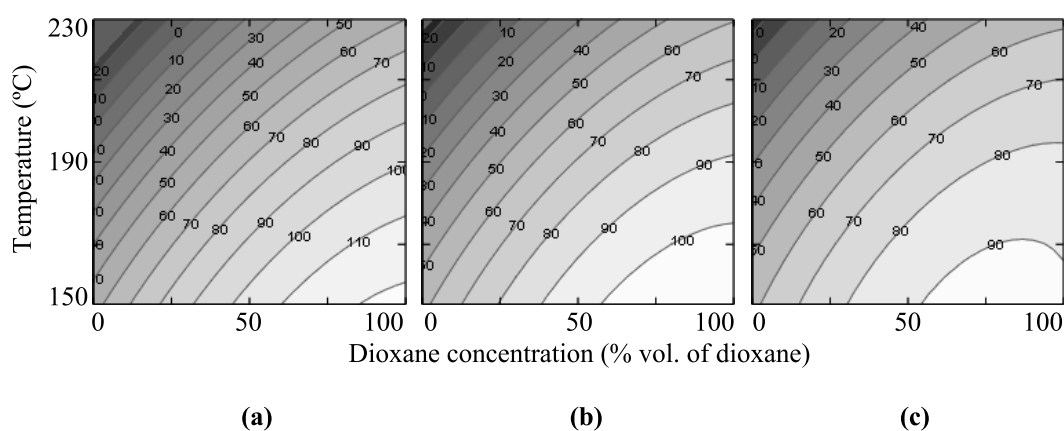
As regards delignification, the dioxane concentration and the extraction temperature had a similar ( $a_1 \approx a_2$ ) and strong influence, while the cooking time again had little effect on this response (low value of  $a_3$ ). Concerning the synergic effect between variables, the dioxane concentration and the extraction temperature showed a high degree of interaction (high value of  $a_{12}$ ), while the synergy between the extraction temperature and the cooking time was moderate

( $a_{23}$ ). There are two appreciable quadratic terms ( $a_{11}$  and  $a_{22}$ ) and, therefore, the predicted surfaces were very steep in the experimental domain, as can also be appreciated from Figure 2; the negative value of the quadratic terms indicates the possible presence of a maximum delignification value within the range of dioxane concentrations and extraction temperatures used. The contour plots for the delignification in the experimental domain of the dioxane concentration and extraction temperature for cooking times of 10, 25 and 40 min are shown in Figure 2. Delignification was zero when pure water was used and high for mixtures containing more than 50% vol. of dioxane at temperatures above 200°C. Delignification passed through a maximum within the dioxane concentrations used, which was greater and moved to increasing dioxane concentrations as the temperature rose. Similarly, this response also passed through a maximum within the range of extraction temperatures used, this maximum being higher and moving towards increasing extraction temperatures as the dioxane concentration rose. The shape of the contour plots was very similar regardless of the cooking time (see Figures 2a, 2b and 2c), the degree of delignification moderately increasing with cooking time. The use of the Solver tool of Microsoft Excel for optimizing the corresponding second order polynomial led to a maximum delignification value of 88.5% when using a dioxane-water mixture of 94% vol. of dioxane at the maximum temperature tested (230°C) and for the longest cooking time (40 min).

Regarding the selectivity of the lignin extraction process, the cooking time practically had no effect on this response (very low value of  $a_3$ ), while the other two independent variables had a similar ( $a_1 \approx a_2$ , in absolute value) and a strong influence; however, the selectivity response surfaces increased as the extraction temperature decreased (negative value of  $a_2$ ) and the dioxane concentration rose (positive value of  $a_1$ ), as can also be appreciated in Figure 3; therefore, temperature was the only parameter that had a very negative effect on selectivity. In this case, the three independent variables interacted moderately. There are two moderately significant quadratic terms ( $a_{11}$  and  $a_{22}$ ) and, therefore, the predicted surfaces were steep in the experimental domain (see Figure 3). Comparison of the contour plots in Figure 3 for cooking times of 10, 25 and 40 min indicated that selectivity slightly decreased with cooking time. The Solver tool of Microsoft Excel pointed to a maximum selectivity of 100% when the extraction was



**Figure 2.** - Predicted contour levels for the delignification (wt%) of HAS as a function of dioxane concentration and extraction temperature for cooking times of: a) 10, b) 25, and c) 40 min.



**Figure 3.** - Predicted contour levels for the selectivity (wt%) of HAS as a function of dioxane concentration and extraction temperature for cooking times of: a) 10, b) 25, and c) 40 min.

carried out with pure dioxane in the mildest conditions used (150°C and 10 min).

The results obtained do not coincide with those reported by Quesada *et al.* [12] applying the classical method of varying one variable at a time to study the organosolv extraction of HAS. This classical experimental design led to a lower maximum delignification degree (87.5% when using a mixture of 75% vol. of dioxane at 210°C and 25 min). Therefore, the use of a second order Doehlert experimental design enabled us to improve the conditions of lignin extraction from HAS and indicated the limitations of the classical design, which does not consider the synergic effect between variables. The fact that the maximum delignification degree obtained by applying the classical design is very similar to that reached with the Doehlert experimental design may be due to the use of starting extraction conditions in the case of the classical design (210°C and 40 min) close to the optimal, which led to a dioxane concentration of 75% vol. The selection of different starting extraction conditions (*e.g.*, a lower temperature) would have led to lower dioxane-to-water ratios and, therefore, to lower maximum delignification degrees (see Figure 2). The maximum delignification degree reached with the dioxane-water mixtures was greater than that obtained with acetone-water and ethanol-water mixtures [12]. Several authors have used aqueous mixtures of dioxane as extracting agent; for example, Barnett *et al.* [13] carried out the extraction of autohydrolyzed poplar wood in a Soxhlet

with an azeotropic dioxane-water mixture for 72 h and were able to reduce the Klason lignin content from 21% to 2.7%. In turn, Beltrame *et al.* [14] achieved a 90% delignification degree during the extraction of autohydrolyzed (230°C for 60 s) wheat straw with a dioxane-water mixture of 50% vol. of dioxane.

The fact that the percentage of dissolved material increases with the severity of the dioxane organosolv extraction (*i.e.*, higher temperatures and longer cooking times) could be explained by the greater degradation of the polymeric structure of the material, which favors the dissolution of the biopolymers in the medium. However, an increase in organosolv extraction severity led to a decrease in the lignin extraction selectivity [15], which was probably due to a greater holocellulose (cellulose in the case of HAS) extraction. Cellulose is more stable than hemicelluloses and lignin [16, 9] due to its microfibrillar structure and crystallinity [17], which hinders its dissolution, so that more severe extraction conditions are needed.

The amount of cellulose dissolved in each assay (see Table 3) was estimated by considering that all the hemicelluloses initially present in HAS (5.9% wt) were extracted in each assay; therefore, since the amount of lignin extracted (delignification) is known, this estimation represents the minimum amount of cellulose which has been extracted. As expected, the greater the severity of the organosolv extraction process, the higher the amount of cellulose extracted and the lower the lignin extraction selectivity. Note that cellulose extraction was only significant when the pro-

cess was carried out at temperatures above 190°C, which would have led to a strong decline in selectivity. In general, lignin is more soluble in organic solvents than in water, while the opposite is true for holocellulose; therefore, the lower the water content of the extraction mixture, the higher its lignin extraction capacity and the lower its holocellulose extraction capacity. For this reason, the maximum degree of delignification was reached when using mixtures with a high dioxane (94% vol.) content, while maximum extractability (lignin and holocellulose together), was attained with a mixture less rich in dioxane (83% vol. of dioxane). If the aim of the organosolv process is to reach the greatest degree of lignin extraction and the highest content of lignin in the extract, it will be necessary to use mixtures with a high dioxane content (>90% vol. of dioxane) to maximize both delignification and selectivity. Subsequently, the extraction temperature and the cooking time will be selected according to the response to be optimized; thus, if the aim is to favor delignification over selectivity, a high extraction temperature (230°C) and long cooking time (40 min) will be necessary, while if a high degree of delignification and an acceptable degree of selectivity are desired, milder extraction conditions should be used.

**Table 3.** - Estimated minimum amount of cellulose extracted during dioxane organosolv extraction of HAS by applying a three-factor Doehlert experimental design.

Essay n°	P (v/v)	T (°C)	t (min)	C (%)	S (%)
1	50/50	190	25	8.2	71.0
2	100/0	190	25	0.0	100.0
3	75/25	230	25	87.1	43.4
4	25/75	230	25	82.5	12.1
5	0/100	190	25	9.2	12.9
6	25/75	150	25	0.0	85.1
7	75/25	150	25	0.0	96.7
8	50/50	190	25	11.8	65.0
9	25/75	203	40	19.6	41.9
10	50/50	163	40	0.0	88.9
11	75/25	203	40	23.6	67.6
12	25/75	177	10	0.0	64.3
13	50/50	217	10	61.7	35.3
14	75/25	177	10	0.0	100.0
15	50/50	190	25	1.1	76.6

P= dioxane-to-water ratio; T= extraction temperature;  
t= cooking time; C= estimated cellulose that  
has been extracted; S= selectivity.

It can be concluded that the most influential variables on the organosolv extraction process were the extraction temperature and dioxane concentration; in the case of delignification and selectivity, both variables had a similar influence, while extractability was most influenced by the extraction temperature. The influence of cooking time was slight in all the cases. Moreover, weak interactions were detected between the dioxane concentration and the cooking time in the case of selectivity and between the dioxane concentration and the extraction temperature in the case of extractability, while a significant interaction between the dioxane concentration and the extraction temperature was obtained in the case of delignification. If (i) the above second order polynomial is derived with respect to the dioxane concentration ( $X_1$ ) [considering that

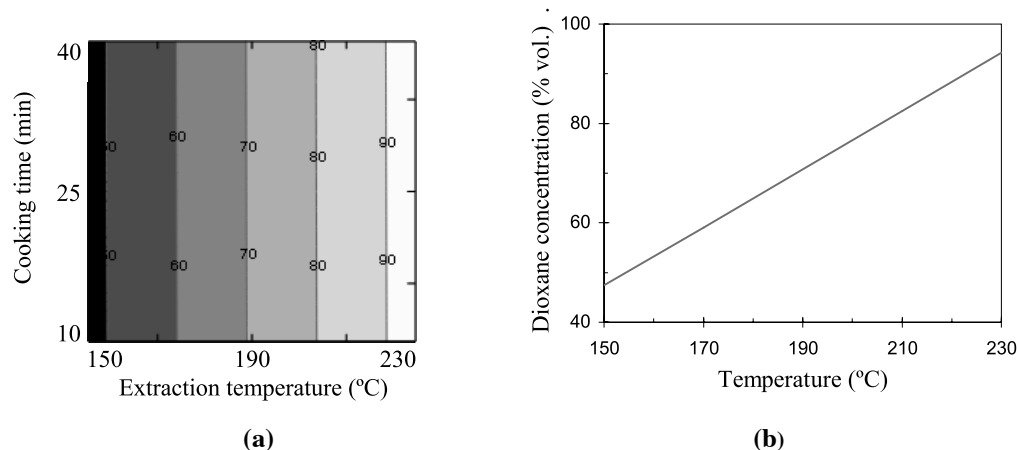
the extraction temperature ( $X_2$ ) and cooking time ( $X_3$ ) are constants], (ii)  $X_1$  is substituted by its value isolated from the expression *Dioxane concentration* = 50 + 50  $X_1$ , (iii) the derived equation is equated to zero, and (iv) we solve for the *Dioxane concentration*, the following linear equation is obtained:

$$\text{Dioxane concentration} = 50 - 25 a_1/a_{11} - (25 a_{12}/a_{11}) X_2 - (25 a_{13}/a_{11}) X_3$$

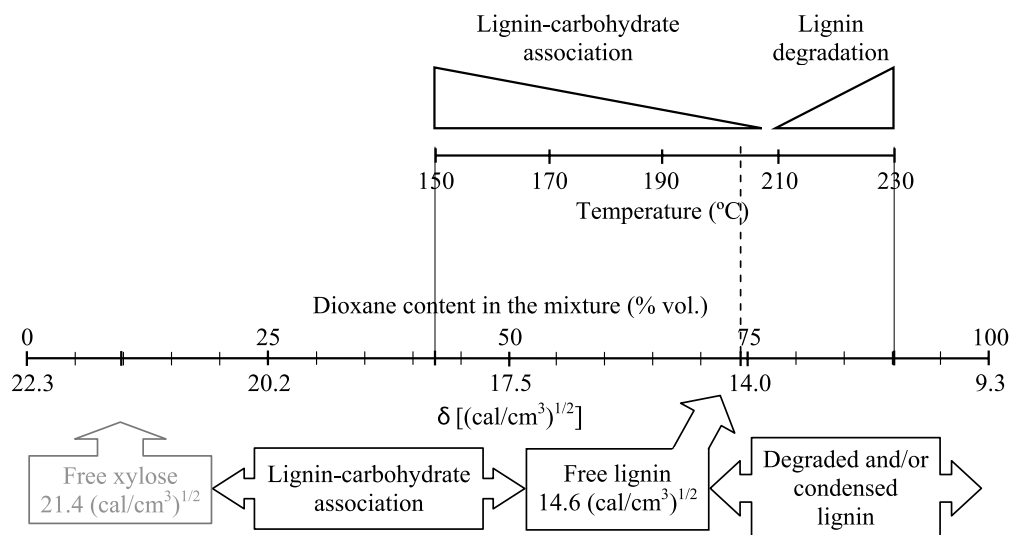
When this expression is particularized for delignification, it determines the optimum dioxane concentration of the solvent mixture as a function of the extraction temperature and cooking time, which leads to the maximum degree of delignification. The contour plot of the above linear equation is shown in Figure 4a. As the cooking time has no influence on the optimum dioxane concentration, the linear relation between the optimum dioxane concentration and the extraction temperature can be represented for, for example, a cooking time of 25 min (see Figure 4b). It can be appreciated that the dioxane concentration of the solvent mixture leading to the maximum degree of delignification increased with extraction temperature, as can also be seen in Figure 2; in this way, the maximum delignification which can be reached at 170°C would be obtained with a mixture of 59% vol. of dioxane, while at 210 and 230°C mixtures of 82% and 94% vol. of dioxane, respectively, would be necessary. Therefore, if a starting temperature of 170°C had been chosen during the application of the same classical method of varying one variable at a time as described by Quesada *et al.* [12], a mixture of 50% vol. of dioxane would have led to maximum delignification. This result would be in apparent conflict with the results predicted by the solubility parameter theory, which postulated that the best mixture is composed of 75% vol. of dioxane [12]. However, as discussed below, the results attained in this study can also be interpreted by means of the solubility parameter theory.

According to this theory [18], the solubility of a polymer in a solvent will be greater, the closer the respective values of their solubility parameters are. Therefore, maximum delignification (lignin solubility) should occur during the organosolv extraction of HAS when the  $\delta$ -value of the solvent mixture is close to that of the lignin to be extracted. If the solubility parameter theory is used to explain the dependence between the optimum dioxane concentration of the mixture leading to the maximum delignification and the extraction temperature, we must take into account that the  $\delta$ -value of the solvent mixture does not depend on the extraction temperature [11]; therefore, the  $\delta$ -value of the lignin extracted probably changes as a result of structural alterations caused by the thermal effect.

As observed in the thioacidolysis analysis and during the determination of Klason lignin of the fractionated organosolv extracts from HAS, the use of low temperatures (150-170°C) causes the extracted lignin to show a high degree of association with saccharides (internal communication). Therefore, because the  $\delta$ -value of saccharides [e.g.,  $\delta_{xylose} = 21.37$  (cal/cm<sup>3</sup>)<sup>1/2</sup>,  $\delta_{glucose} = 22.42$  (cal/cm<sup>3</sup>)<sup>1/2</sup>] is greater than that of lignin [14.60 (cal/cm<sup>3</sup>)<sup>1/2</sup>], the  $\delta$ -value of the lignin associated to carbohydrates (lignin-carbohydrate complexes) will be higher than that of the free lignin and close to that of the dioxane-water mixtures [ $\delta_{dioxane} = 9.30$  (cal/cm<sup>3</sup>)<sup>1/2</sup>,  $\delta_{water} = 22.31$  (cal/cm<sup>3</sup>)<sup>1/2</sup>] [12], which thus be-



**Figure 4.-** Dioxane concentration (vol.%) of the solvent mixture that maximizes the organo-solv delignification of HAS: a) contour plot in the domain of the extraction temperature and cooking time, b) as a function of the extraction temperature for a cooking time of 25 min.



**Figure 5.-** General schema of the delignification process from hydrolyzed almond shells using dioxane–water mixtures. Influence of the extraction temperature on the dioxane concentration that maximizes the delignification and interpretation by means of the solubility parameter theory.

come the most suitable for dissolving these complexes; that is, although free lignin is dissolved with more difficulty in water-rich mixtures, these mixtures have a much greater capacity to dissolve the lignin-carbohydrate complexes, achieving generally higher delignification degrees. Therefore, the lower the extraction temperature, the higher the water content of the solvent mixture to maximize delignification, since the degree of association between the lignin extracted and saccharides is greater. Lignin-carbohydrate associations may be of the covalent type through ester [19] and/or ether (benzylic-type [20], phenyl-type [21] and/or acetal-type [22]) bonds, forming the lignin-carbohydrate complexes, and/or of a non-covalent type by means of electrostatic forces between the lignin and cellulose [23]. Lawoko [24] claimed that almost all the lignin in softwood is associated with carbohydrates, forming the lignin-carbohydrate complex. In the case of almond shells, Caballero *et al.* [25] confirmed, by thermal analysis (TG/DTA), that there is a clear interaction between lignin and holocellulose; these authors observed different thermal decomposition behaviour between the isolated biopolymers (lignin

and holocellulose separately) and the biopolymers inside the non-fractionated material (lignin and holocellulose together inside the lignocellulosic matrix). Moreover, the use of moderate temperatures (200-210°C) causes the association between lignin and holocellulose to practically disappear (internal communication). In this way, the dioxane–water mixture that would lead to the maximum delignification degree at this temperature should have a  $\delta$ -value very close to that of free lignin [14.60 (cal/cm<sup>3</sup>)<sup>1/2</sup>], that is, it should be the mixture with a 75% vol. of dioxane [ $\delta$ -value = 14.03 (cal/cm<sup>3</sup>)<sup>1/2</sup>], which has been demonstrated experimentally [12]. If temperatures above 200-210°C are used, the best mixtures for extracting lignin are composed of more than 75% vol. of dioxane: the higher the temperature, the greater the dioxane content of the mixture. As the mixtures with a very high dioxane content have  $\delta$ -values very close to that of pure dioxane [9.30 (cal/cm<sup>3</sup>)<sup>1/2</sup>], the  $\delta$ -value of the lignin extracted in these conditions should be close to 9.30 and, therefore, lower than that of free lignin. The thermal effect

of temperatures above 210°C may cause condensation and degradation of the lignin structure, resulting in products with a  $\delta$ -value lower than that of free lignin. Among degradation and condensation [26] reactions are the dehydration of the phenylpropane units [27], the formation of diphenylmethane-type compounds [28] and the propane chain cleavage of the phenylpropane units leading to compounds such as vanillin and syringaldehyde [27, 29, 30]. The diphenylmethane-type compounds, vanillin [ $\delta$ -value = 10.80 (cal/cm<sup>3</sup>)<sup>1/2</sup>] and syringaldehyde [ $\delta$ -value = 10.48 (cal/cm<sup>3</sup>)<sup>1/2</sup>], as well as 3,5-di-tert-butyl-4-hydroxytoluene and 2,4-di-tert-butylphenol [highly condensed and branched compounds with  $\delta$ -values of 9.04 y 9.05 (cal/cm<sup>3</sup>)<sup>1/2</sup>, respectively] were detected by GC-MS in the organosolv extracts from HAS obtained at 230°C and have  $\delta$ -values close to that of pure dioxane.

A general schema of the chemical phenomena that occurred during the delignification process of hydrolyzed almond shells using dioxane–water mixtures is presented in Figure 5; moreover, the application of the solubility parameter theory to explain lignin extraction from HAS is also included in this figure.

The  $\delta$ -values of free lignin, free saccharides and lignin-carbohydrate complexes are located in this schema on a scale of solubility parameters for binary dioxane–water mixtures, illustrating the dependence between the extraction temperature, the dioxane concentration of the mixture that maximizes delignification, and the degree of association between lignin and carbohydrates and of degradation of the lignin extracted. Thus, the mixture leading to the highest delignification degree at 150°C was composed of a 42% vol. of dioxane, and, under these conditions, the lignin-carbohydrate complexes were mainly extracted. As the temperature increased, the association between lignin and carbohydrates decreased and, at around 200-210°C, this association virtually disappeared. Therefore, at 200-210°C the lignin was not associated with carbohydrates (free lignin) and was extracted better in mixtures with  $\delta$ -values close to that of lignin [ $\delta$ -value = 14.6 (cal/cm<sup>3</sup>)<sup>1/2</sup>], i.e., in mixtures with around a 75% vol. of dioxane [ $\delta$ -value = 14.0 (cal/cm<sup>3</sup>)<sup>1/2</sup>], which was observed experimentally [12]. At above 200-210°C the lignin structure began to condense and degrade: the higher the extraction temperature, the greater the extent of these reactions leading to dehydrated, broken and condensed compounds based on phenylpropane units, whose  $\delta$ -values are lower than that of free lignin and therefore dissolve better in mixtures rich in dioxane (>75% vol. of dioxane); in this way, the best mixture obtained at 230°C was composed of a 90-95% vol. of dioxane.

#### 4. CONCLUSIONS

The use of a three-factor Doehlert experimental design for the non-catalyzed dioxane organosolv extraction of lignin from hydrolyzed almond shells showed that the dioxane concentration of the extraction mixture leading to a maximum delignification degree depends on the extraction temperature: the higher the extraction temperature, the greater the dioxane concentration of the mixture needed. This behaviour can be explained by means of the solubility parameter theory and taking into account that the temperature affects both the degree of association between the

lignin extracted and carbohydrates, and the degradation of the lignin structure.

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#### BIBLIOGRAPHY

1. Xu, Y.J., Li, K.C., Zhang, M.Y., 2007. Lignin precipitation on the pulp fibers in the ethanol-based organosolv pulping. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 301, 255-263.
2. Rodríguez, A., Jiménez, L., 2008. Pulping with organic solvents other than alcohols. *Afinidad* 65 (535), 188-196.
3. Jiménez, L., Rodríguez, A., Pérez, A., Moral, A., Serano, L., 2008. Alternative raw materials and pulping process using clean technologies. *Ind. Crop. Prod.* 28, 11-16.
4. Pan, X.J., Xie, D., Yu, R.W., Saddler, J.N., 2008. The bioconversion of mountain pine beetle-killed lodgepole pine to fuel ethanol using the organosolv process. *Biotechnol. Bioeng.* 101, 39-48.
5. Paszner, L., Cho, H.J., 1989. Organosolv pulping: acidic catalysis options and their effect on fiber quality and delignification. *Tappi J.* 72, 135-142.
6. López, F., García, J.C., Pérez, A., García, M.M., Feria, M.J., Tapias, R., 2010. *Leucaena diversifolia* a new raw material for paper production by soda-ethanol pulping process. *Chem. Eng. Res. Des.* 88, 1-9.
7. Rodríguez, A., Sánchez, R., Ferrer, A., Requejo, A., 2011. Simulation of *Hesperaloe funifera* diethanolamine pulping by polynomial and neural fuzzy models. *Chem. Eng. Res. Des.* 89, 648-656.
8. López, F., Pérez, A., García, J.C., Feria, M.J., García, M.M., Fernández, M., 2011. Cellulosic pulp from *Leucaena diversifolia* by soda-ethanol pulping process. *Chem. Eng. J.* 166, 22-29.
9. Martínez, J.M., Granado, J.M., Montané, D., Salvadó, J., Farriol, X., 1995. Fractionation of residual lignocellulosics by dilute-acid prehydrolysis and alkaline extraction: application to almond shells. *Bioresour. Technol.* 52, 59-67.
10. Quesada, J., Teffo-Bertaud, F., Croué, J.P., Rubio, M., 2002. Ozone oxidation and structural features of an almond shell lignin remaining after furfural manufacture. *Holzforsch.* 56, 32-38.
11. Quesada, J., Rubio, M., Gómez, D., 1998. Lignin organosolvolytic from autohydrolyzed corn stalks: ozonation of both solvolytic and juice. *J. Appl. Polym. Sci.* 68, 1867-1876.
12. Quesada-Medina, J., López-Cremades, F.J., Olivares-Carrillo, P., 2010. Organosolv extraction of lignin from hydrolyzed almond shells and application of the  $\delta$ -value theory. *Bioresour. Technol.* 101, 8252-8260.
13. Barnet, D., Excoffier, G., Vignon, M., 1989. Valorisation de la biomasse lignocellulosique: autohydrolyse rapide de copeaux de bois de peuplier. *Bull. Soc. Chim. Fr.* 6, 836-843.



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14. Beltrame, P.L., Carniti, P., Visciglio, A., Focher, B., Marzetti, A., 1992. Fractionation of steam-exploded wheat straw. *Bioresour. Technol.* 39, 165-171.
  15. Vázquez, G., Antorrena, G., González, J., Freire, S., López, S., 1997. Acetosolv pulping of pine wood. Kinetic modelling of lignin solubilization and condensation. *Bioresour. Technol.* 59, 121-127.
  16. Melcer, I., Melcerova, A., Kacik, F., Sindler, J., Vargicova, K., Sertic, V., 1990. Changes in the properties of beech wood. *Drevo (Praha tren Bratislava)* 45, 255-260.
  17. Li, J., Henriksson, G., Gellerstedt, G., 2005. Carbohydrate reactions during high-temperature steam treatment of aspen wood. *Appl. Biochem. Biotechnol.* 125, 175-188.
  18. Hildebrand, J.H., Scott, R.L., 1950. The solubility of nonelectrolytes, third ed. Rheinhold, New York.
  19. Watanabe, T., Koshijima, T., 1988. Evidence for an ester linkage between lignin and glucuronic acid in lignin-carbohydrate complexes by DDQ-oxidation. *Agric. Biol. Chem.* 52, 2953-2955.
  20. Watanabe, T., 1989. Structural studies on the covalent bonds between lignin and carbohydrate in lignin-carbohydrate complexes by selective oxidation of the lignin with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. *Wood Res.* 76, 59-123.
  21. Kondo, T., Mizuno, K., Kato, T., 1990. Cell wall bound p-coumaric and ferulic acids in italian regrass. *Can. J. Plant Sci.* 71, 495-499.
  22. Xie, Y., Yasuda, S., Wu, H., Liu, H., 2000. Analysis of the structure of lignin-carbohydrate complexes by specific <sup>13</sup>C tracer method. *J. Wood Sci.* 46, 130-136.
  23. Houtman, C.J., Atalla, R.H., 1995. Cellulose-lignin interactions. *Plant Physiol.* 107, 977-984.
  24. Lawoko, M., 2005. Lignin polysaccharide networks in softwood and chemical pulps: characterisation, structure and reactivity. Ph.D. Thesis, Royal Institute of Technology (KTH), Stockholm, Sweden.
  25. Caballero, J.A., Font, R., Marcilla, A., 1996. Comparative study of the pyrolysis of almond shells and their fractions, holocellulose and lignin. Product yields and kinetics. *Thermochim. Acta* 276, 57-77.
  26. Hage, R.E., Brosse, N., Sannigrahi, P., Ragauskas, A., 2010. Effects of process severity on the chemical structure of *Miscanthus* ethanol organosolv lignin. *Polym. Degrad. Stab.* 95, 997-1003.
  27. LeVan, S.L., 1989. Thermal degradation. In: Schniewind, Arno P. (Eds.), *Concise Encyclopedia of Wood & Wood-Based Materials*. Pergamon Press, New York, pp. 271-273.
  28. Argyropoulos, D.S., Ahvazi, B.C., Pageau, G., Liu, Y., 1999. Lignin condensation reactions during pulping and oxygen delignification. 6th Brazilian Symposium on the Chemistry of Lignins and Other Wood Components. Guaratinguetá, SP, Brazil.
  29. Lindner, A., Wegwerner, G., 1990. Characterization of lignins from organosolv pulping according to the Organocell process. Part 3. Molecular weight determination and investigation of fractions isolated by GPC. *J. Wood Chem. Technol.* 10, 331-350.
  30. Ucar, G., Meier, D., Faix, O., Wegener, G., 2005. Analytical pyrolysis and FTIR spectroscopy of fossil sequoiadendron giganteum (Lindl.) wood and MWLs isolated hereof. *Holz. Roh. Werkst.* 63, 57-63.