PHYSICO-CHEMICAL CHARACTERIZATION OF POLYSACCHARIDES AND EXTRACTION OF CELLULOSE FROM ANNUAL AGRICULTURAL WASTES

IBTISSEM MOUSSA,[*,**] RAMZI KHIARI,[*,***,****] ALI MOUSSA,[**,*****]
MOHAMED FAROUK MHENNI[*] and MOHAMED NACEUR BELGACEM[****]

[*]*University of Monastir, Faculty of Sciences, UR13 ES 63 – Research Unit of Applied Chemistry and Environment, 5000 Monastir, Tunisia*
[**]*University of Monastir, National Engineering School of Monastir, 5019 Monastir, Tunisia*
[***]*Higher Institute of Technological Studies of Ksar Hellal, Department of Textiles, Tunisia*
[****]*University of Grenoble Alpes, CNRS, Grenoble INP, LGP2, F-38000 Grenoble, France*
[*****]*University of Monastir, Textile Engineering Laboratory, Tunisia*
✉ *Corresponding author: R. Khiari, Khiari_ramzi2000@yahoo.fr*

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In Tunisia, important quantities of almond and fig stems are accumulated each year. An approach to valorizing these renewable resources has been proposed in this study. This work is focused on the chemical composition of almond and fig stems. The polysaccharide content for both materials was determined to be of about 60%. That is the main reason that justifies their pulping. The sugar composition of almond and fig stem waste consisted of glucose and xylose, and minor quantities of galactose, arabinose and mannose. In this study, single cellulose fibres were extracted from almond and fig stems using chemical treatment. The extraction with soda-anthraquinone delignification, which is the simplest alkaline pulping process, was followed by bleaching. Moreover, the optimization of cellulose extraction was done using the Box-Behnken experimental design. To characterize the ensuing fibres, several methods were used.

Keywords: almond stem waste, fig stem waste, cellulose fibre, Box-Behnken Design (BBD), pulp yield, residual lignin

INTRODUCTION

Over the last decade, the consumption of lignocellulosic materials has increased by 50%.[1] The growing textile and paper production was accompanied by a demand for new categories and technological developments in agreement with environmental protection.[2] The rising demand for fibres requires increased forestry production, which may lead to global deforestation, with harmful consequences to the environment. Because of growing fibre concerns and the potential increase in wood fibre costs, non-wood materials, such as annual plants, have received more attention in the production of pulp, paper, cellulose derivatives and composites.[3–5] Such raw materials offer at least three advantages in the production of pulp.[2] First, the pulp production time is shorter for annual plants than for wood. Second, the consumption of chemicals necessary for cellulose extraction from annual plants is lower than in the case of wood. Third, the bleaching process used for non-wood fibers is simple.

Lignocellulosic wastes are used as animal feed or burnt or left to decompose in the soil.[6] However, they can be converted into a variety of chemicals, such as alcohols, enzymes, organic acids, vitamins, polymers *etc*., thanks to their lignocellulosic structure (cellulose (35-50%), hemicellulose (20-35%) and lignin (5-30%)).[7] The utilization of these cheap and widely disposable wastes does not only resolve environmental pollution, but also gives additional value. The valorization of fibres isolated from Tunisian agricultural residues and annual plants (date palm rachis, alfa stems, vine stems, olive stones, *Posidonia oceanica*, *Juncus*, bagasse and *Tamarisk sp*.) has been investigated for producing paper, green composites and cellulose

derivatives.[3–5,8–14] In this study, two lignocellulosic materials largely available in Tunisia were investigated as a source of cellulosic fibres, namely almond and fig stems.

Almond stems are a by-product commonly considered useless, being incinerated or dumped. According to the Food and Agricultural Organization (FAO), Tunisia is ranked as the 8th producing country of almond with about 3.8% of the total world production.[15] The main production area is located in the coastal regions (Bizerte, Cap Bon, Mahdia, Sfax, Zarzis, Djerba, Kerkenah *etc*.) and in some mountainous regions (Gafsa, Beni-Khedache *etc*.). As for fig stems, no data about their chemical composition are available in the literature. This paper aims at contributing to a deeper understanding of the chemical and structural composition of these wastes and their cellulosic fibres. The first part of this work investigates the chemical composition of these two agricultural residues, which are compared to other biomass types, such as wood and non-wood sources. Pulping of these residues was accomplished using the soda-anthraquinone process, which is the most appropriate process for pulping agricultural wastes.[16] In the second part of this study, the effect of the extraction conditions was analyzed and optimized using the Box-Behnken experimental design. Three factors of the pulping process were optimized, namely the temperature, sodium hydroxide concentration and reaction time. Moreover, the bleaching steps were also optimized with regard to the pH, temperature, concentration of the hypochlorite sodium and reaction time.

EXPERIMENTAL

Preparation of materials

Almond and fig stem waste was collected from Monastir (Tunisia) in August 2017. It was washed to remove impurities and ensure reliable determination of the chemical composition. Then, the stems were dried under natural conditions in the month of September 2017. After that, as recommended by the standard method,[17] our materials were milled and sieved to a particle size between 200 and 400 µm.

Chemical composition

The chemical composition of the raw materials was established using different standards or methods, as summarized in Table 1. The evaluation of extractives was carried out in different liquids, according to common standards, namely, cold and hot water solubility (T207 cm-08), 1% sodium hydroxide solution solubility (T212 om-07), and ethanol–toluene solubility (T204 cm-07). The ash content was determined according to the standard procedure TAPPI T211 om-07, by calcination of the materials at 525 ± 25 °C for at least 4 hours. The amounts of lignin and α-cellulose were also established by using the following TAPPI methods: T222 cm-99 and T203 cm-99, respectively. Finally, the holocellulose content was determined according to the method described by Wise *et al*.[18]

Monosaccharide composition of raw materials

Content of total sugars

The content of total sugars was determined according to the method of Dubois *et al*.[19] The method is based on the colour reaction between phenol, concentrated H_2SO_4 and sample sugars, oligosaccharides, polysaccharides and their derivatives, including methyl esters, which contain a free or potentially free reducing group. The method is applicable to 2 mL test solution containing from 10 to 70 µg sugar. A yellow-orange colour is produced between 25 and 30 °C, and the absorption is measured at 490 nm for hexoses and 480 nm for pentoses and uronic acids.

Acid hydrolysis and ion chromatography

Ion chromatography is an analytical technique that provides the best recovery of sugars with minimum hydrolysis of sucrose. The analytical hydrolysis procedure uses a two-step acid hydrolysis.[20] Primary hydrolysis of 350 mg sample was performed with 3 mL 72% (w/w) H_2SO_4 for 1 hour at 30 °C. The hydrolysates were diluted to 4% (w/w) H_2SO_4 with distilled water. A secondary hydrolysis was performed for 1 hour at 120 °C in an autoclave (1 bar) to fractionate the biomass into forms that are more easily quantified. Fucose was added as an internal standard. The hydrolysates were diluted to 200 mL with H_2O and filtered through a 0.45 µm Teflon syringe filter. Then, 10 µL of hydrolysates were injected into the chromatographic system with no additional treatment. The calibration, which was based on a standard mixture of sugars treated in parallel with each sample, was used to correct the degradation of sugars during secondary hydrolysis. Losses during primary hydrolysis were minimal and were ignored. The sugar contents of the hydrolysates were determined by ion chromatography.

Pulping by the soda-anthraquinone process

Pulping of the agricultural wastes was carried out using the soda-anthraquinone process. Various parameters affect the quality of the obtained fibres in terms of kappa number and pulp yield, namely, temperature, time and sodium hydroxide concentration. In order to assess their effects, experiments were carried out using the Box-Behnken experimental design (BBD).[21] Each parameter was varied across three levels (-1, 0 and +1), as listed in Table 2. The

range of the analysed factors was chosen based on studies reported in the literature and the authors' experience. BBD involves 15 experimental sets and each experimental set is carried out three times. It is a statistical approach to estimate the effect of each factor and to determinate the optimum conditions for the process. The results of BBD were analysed to model the response as a function of the influencing factors and their interactions, following a second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i,j=1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (1)$$

where Y is the predicted response, (β_0, β_1, β_2 and β_3) are coefficients of the linear model, (β_{11}, β_{22} and β_{33}) are coefficients of the square part of the model, (β_{12}, β_{13} and β_{23}) are coefficients of the 2-way interaction terms, X_1, X_2 and X_3 are coded independent variables. The statistical and graphical analyses were made using Minitab software (MINITAB®17.1).

Table 1
Chemical composition of almond and fig stems

Components			Values	
			Almond stems	Fig stems
Humidity (%)			7.65±2.52	7.85±2.62
Cold water extractives (%)			12.17±3.10	9.24±3.32
Hot water extractives (%)			16.70±4.19	12.70±4.27
Ethanol–toluene extractives (%)			7.02±3.69	4.18±3.08
1% NaOH extractives (%)			29.01±4.12	21.57±4.24
Ash (%)			3.39±1.47	5.10±1.52
Klason lignin (%)			34.35±3.22	19.64±3.16
Soluble lignin (%)			0.76±0.11	1.08±0.13
Holocellulose (%)			50.66±2.57	60.11±2.17
Hemicellulose (%)	TAPPI method		19.25±1.44	13.05±1.25
	Ion chromatography (%)		18.61	15.62
α-Cellulose (%)	TAPPI method		31.41±3.79	47.06±3.24
	Ion chromatography (%)		33.60	49.33
Sugar composition (%)	Ion chromatography (%)	Glucose (%)	29.77	44.17
		Xylose (%)	12.53	14.29
		Galactose (%)	6.79	3.25
		Arabinose (%)	3.13	1.95
		Mannose (%)	0	1.30
		Total	52.22	64.96
	Dubois Dosage (%)		46.58±2.45	58.41±2.17
Total charge (μeq.g^{-1})			80.00±1.25	110.00±1.09

Table 2
Factors and levels of the Box-Behnken experimental design for the soda-anthraquinone and bleaching process

Factors	Symbols	Levels		
		-1	0	1
Delignification steps				
Temperature (°C)	X_1	80	110	140
Time (min)	X_2	60	120	180
Sodium hydroxide concentration (%)	X_3	8	12	16
Bleaching step				
pH	X_1	9	11	13
Temperature (°C)	X_2	30	45	60
Sodium hypochlorite concentration (%)	X_3	30	50	70
Time (min)	X_4	60	120	180

After pulping, the obtained fibres were separated from the black liquor. The kappa number was determined using TAPPI Method T236om06. The kappa number is the volume (in millilitres) of the 0.1 mol.L^{-1} potassium permanganate solution consumed by one gram of moisture-free pulp under the conditions

specified in this method. The results were corrected to 50% consumption of the permanganate added.

Bleaching process

Following the soda-anthraquinone treatment under optimum conditions, the bleaching process was completed by adding a solution of soda (pH 9-13), sodium hypochlorite (30-70%) at 30-60 °C for 60-180 min. The mixture was filtered and purified by an anti-chlorine treatment. Sodium hypochlorite bleaching must always be completed with an anti-chlorine treatment. Anti-chlorine is a neutralization treatment, which is performed to eliminate the excess chlorite. An antichlor is a substance used to decompose residual hypochlorite or chlorine after chlorine-based bleaching, in order to prevent ongoing reactions with it, which cause damage to the material that has been bleached.

In this section, a second Box-Behnken experimental design was used to investigate the optimal conditions of the bleaching treatment, as shown in Table 2. The system behaviour was explained by a quadratic model equation, which includes the effects of linear, quadratic and 2-way interaction terms. This equation was used to determine the predicted response as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i,j=1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \quad (2)$$

where Y is the predicted response, (β_0, β_1, β_2, β_3 and β_4) are coefficients of the linear model, (β_{11}, β_{22}, β_{33} and β_{44}) are coefficients of the square part of the model, (β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34}) are coefficients of the 2-way interaction terms, and X_1, X_2, X_3 and X_4 correspond to the principal factors of pH, temperature, concentration of sodium hypochlorite and time, respectively.

Characterization

Total charge: conductometric titration

To determine the total charge of the raw materials, we used the procedure described by Katz *et al.*[22] and Fras *et al.*[23] An amount of 1 g oven-dry samples was added to an airtight titration vessel. 500 mL distilled water and 1 mL 0.5 mol.L^{-1} NaCl were added to the vessel to obtain a 1 mol.L^{-1}NaCl solution concentration during titration. Furthermore, 0.5 mL HCl (0.1 mol.L^{-1}) was added to the vessel. During titration, the solution was stirred with a magnetic stirrer at 25 °C. The titration was performed by adding 0.1 mol.L^{-1} NaOH, using a precision burette, in drops of 0.04 mL, with 1 min intervals. Conductivity was recorded with a conductometer. The titration was finished at a pH of ≈10.5, which makes proper extrapolation of the intersection/end point possible. A blank titration without samples was performed to calibrate the system and to eliminate the effects of impurities. The total charge was then obtained by extrapolating the second and the third linear parts of the titration curve until they intersect. It was calculated using the following equation:

$$\text{Total charge } (\mu\text{eq.g}^{-1}) = (C_{OH}.V_t) / m_{dry} \quad (3)$$

where C_{OH} is the concentration of the sodium hydroxide solution, V_t is the volume of the sodium hydroxide solution consumed at the second intersection point and m_{dry} is the oven-dry weight of sample.

Morphological analysis

A Scanning Electron Microscope (SEM) and a Morfi (LB-01) analyzer (Techpap, France)[19] were used to study the morphology of the fibres. The fibre parameters were measured by image analysis of a diluted suspension captured by a CCD video-camera. The average length, width and the proportion of fine elements were measured and evaluated.

Gel permeation chromatography analysis

The weight average molecular weights were established by gel permeation chromatography (CPG) after tri-carbanilation of cellulose. Cellulose (15 mg) was put in a test tube, equipped with micro stir bars, and dried overnight under vacuum at 40 °C. After the addition of anhydrous pyridine (4 mL) and phenyl isocyanate (0.5 mL), the mixture was stirred for 48 hours at 70 °C. Then, methanol (1 mL) was added to quench the phenyl isocyanate. Next, the content of the test tube was added to 7:3 (v:v) methanol:water (100 mL) to precipitate the derivatized cellulose. Finally, the solid was filtrated, washed with the same methanol:water solution, followed by washing with deionized water, and then dried overnight under vacuum at 40 °C. The derivatized cellulose was dissolved in tetrahydrofuran (THF) (1 mg.mL^{-1}), filtered through a 0.45 mm filter and placed in a 2 mL auto-sampler vial. The molecular weight distributions of the cellulose tricarbanilate samples were analysed on an Agilent GPC security 1200 system. Molecular weight was calculated by the software relative to the polystyrene calibration curve. The weight average degree of polymerization (DP_w) was obtained by dividing M_w by the molecular weight of the tri-carbanilated cellulose repeat unit (519 g.mol^{-1}).

XRD analysis

The XRD of the materials was established using X-ray diffraction. The samples were examined using an X-ray diffractometer (D8-Advance Bruker AXS GmbH) at room temperature with a monochromatic CuK_α radiation source (λ = 0.154 nm) in the step-scan mode with a 2θ angle ranging from 5° to 60°, with a step of 0.04° and scanning time of 5.0 min. The crystallinity index (CI) of the sample was determined according to the method described by Segal *et al.*:[24]

$$CI = [(I_{200} - I_{am}) / I_{200}] * 100 \quad (4)$$

where I_{200} is the intensity of the peak in the crystalline plan 200 ($2\theta = 22.6°$) and I_{am} is the intensity of the peak in the amorphous portion ($2\theta = 19.0°$).

RESULTS AND DISCUSSION

Characterization of raw materials

Table 1 shows the average results of the chemical analysis of the fig and almond stem wastes. The obtained results indicate that the raw materials are characterized by relatively large amounts of extractives (more than 12% in hot water). The main difference between the investigated materials is related to the ash amount, which is higher in the case of the fig stems, *i.e.*, 5.10%, than that of the almond stems (3.39%). The extractions carried out under alkaline conditions yielded very high content, more than 20%, probably representing oligosaccharide and lignin-rich materials. Polysaccharides are the major component of almond and fig stems, with average values of 52.22% and 64.96%, respectively. The monosaccharide compositions are also given in Table 1. As may be noted, there is no significant difference in the monosaccharide composition. Almond and fig stems mainly contained glucose and xylose. Galactose, arabinose and mannose were present in relatively minor quantities. Based on the results of the current study, it can be concluded that there is a significant difference in the yield of polysaccharides and lignin contents of the almond and fig stems. In conclusion, the almond and fig stem wastes are characterized by large amounts of cellulose. The cellulose content of the fig stems (47%) was close to those of hardwoods and softwoods, such as *Eucalyptus globulus*, *Pinus pinaster*, olive trimmings and higher annual and perennial plants.[25,26]

Analysis of the pulping treatment

For the two Tunisian agricultural wastes, the pulping treatment was evaluated by varying the soda-anthraquinone process parameters, namely temperature, time and sodium hydroxide concentration. The experiments were carried out according to the three levels Box-Behnken experimental design presented in Table 2. The obtained results in terms of pulp yield and kappa number were summarized in Table 3.

Main effects plot

Figure 1 shows the main effects plot for pulp yield and kappa number. It should be noted that the steeper the slope of the line, the greater the magnitude of the main effect. The results obtained for the two studied materials revealed that temperature is the factor that has the most significant effect on the responses. The higher the temperature, the smaller the pulp yield and the kappa number of the samples, while a smaller effect is exhibited by the sodium hydroxide concentration. However, a few variations were caused by the pulping time.

Table 3
Box-Behnken experimental design for the soda-anthraquinone process

Expt. N°	Factors			Responses			
				Almond stems		Fig stems	
	X_1	X_2	X_3	Yield (%)	Kappa	Yield (%)	Kappa
1	-1	-1	0	54.42	54.03	71.46	62.18
2	0	-1	1	51.01	48.27	68.33	65.27
3	1	0	1	47.37	47.38	64.25	54.56
4	0	0	0	44.42	41.42	60.16	42.10
5	0	-1	-1	40.30	38.09	57.67	47.39
6	0	1	-1	45.26	47.02	62.29	44.94
7	1	-1	0	45.94	47.42	63.38	55.26
8	-1	0	1	51.47	47.51	68.77	57.08
9	1	1	0	44.53	39.05	60.43	45.02
10	-1	1	0	47.09	47.16	64.21	54.34
11	-1	0	-1	48.17	47.01	65.44	60.55
12	1	0	-1	46.34	41.08	64.22	53.88
13	0	0	0	52.19	55.93	68.86	60.50
14	0	0	0	46.83	46.72	64.37	53.91
15	0	1	1	52.10	48.66	68.93	57.48

X_1: Temperature (°C); X_2: Time (min); X_3: Sodium hydroxide concentration (%)

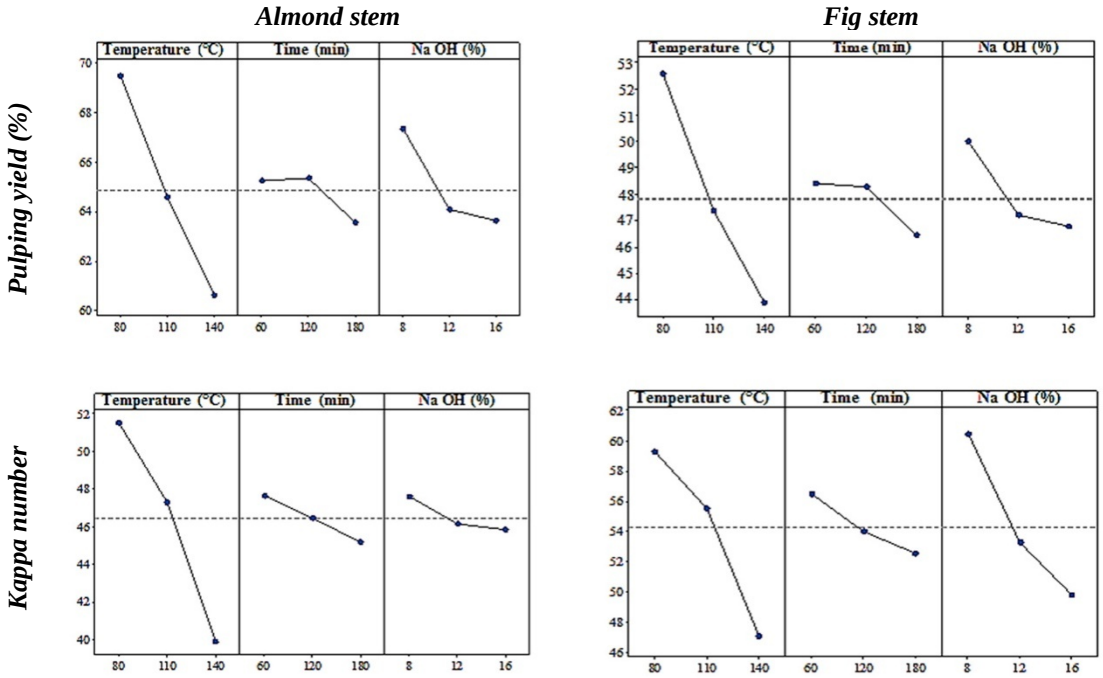

Figure 1: Main effects plot of pulping parameters for pulping yield and kappa number of almond and fig stems

Based on these main effects plot (Fig. 1), several conclusions can be drawn. (i) Pulping temperature: as the temperature was increased from 80 to 140 °C, there was a significant decrease in the pulp yield and residual lignin.

The lignins were dissolved at different speeds during cooking and these rates significantly accelerated upon increasing temperature. (ii) Pulping time: for each starting material, the cooking time slightly affects the quality of the pulp. In fact, when the time of pulping is longer, the delignification follows the same trend and consequently the content of lignin seems to be lower, as well as the pulping yield. (iii) Sodium hydroxide concentration: when the sodium hydroxide concentration increases from 8 to 12%, the pulping yield and the amount of lignin decrease significantly, whereas when the NaOH concentration increases between 12 and 16%, the pulping yield and the amount of lignin decrease slightly.

Fitted models and optimization of pulping parameters

Statistical analysis of the experimental results from Table 3 was performed using Minitab software. The objective is to model the responses (the pulping yield and kappa number) as a function of the process parameters (temperature, time and sodium hydroxide concentration) and their interactions. This allows predicting pulp yield and kappa number according to the studied parameters. The coefficients of quadratic models (according to Eq. 1) fitted for the two different raw materials are shown in Table 4. With high values of the determination coefficients (R^2 > 94% and R^2 (adj) > 84%), the obtained models can be considered as good statistical models with high correlation between the observed and the predicted values. It should be noticed that the closer the value of R^2 to unity, the better the empirical models fit the actual data within the range of the chosen variables. For the two different waste materials, the temperature seems to have a considerable effect on the amount of residual lignin. The optimal process conditions obtained for maximizing the production of pure fibre cellulose from the investigated wastes are as follows: in the case of almond stems – temperature of 120 °C, time of 70 min and NaOH concentration of 9%, while in the case on fig stems – temperature of 135 °C, time of 75 min and NaOH concentration of 15%. These conditions were optimized using the quadratic models shown in Table 4. To validate the predicted values for these optimal process conditions, experiments were carried out under the same conditions and the obtained values were compared with the predicted ones. The results were presented in Table 4. It can be observed that

the predicted and experimental values are in good agreement, showing differences smaller than 5%. This proves that the fitted Box-Behnken models are reliable, with a good degree of accuracy, within the range of the experimental conditions.

Analysis of bleaching treatment

In this part of the study, the effects of bleaching parameters, namely pH, time, NaOCl concentration and temperature, were examined in order to establish the best conditions. The results were tabulated in Table 5. Hence, further analysis was conducted to study the influence of the extraction process parameters on the yield and kappa number. The main effects plots of the bleaching parameters for both materials were regrouped in Figure 2. It can be noticed that the pH is a very important variable for the decomposition of NaOCl, which is the most significant element in the bleaching steps. The proportions of chlorine, hypochlorous acid and hypochlorite in the solution depend on the pH. The hypochlorous acid is considered destructive to the cellulose and therefore bleaching is avoided in the pH range from 2 to 9. It is essential to maintain the pH at a suitable level for a hypochlorite bleach to prevent the attack of the cellulose and thus enough soda should be added to be certain that the final pH will be equal to at least 9. Hydrochloric acid must be neutralized to prevent the pH from dropping. For each starting material, the pH improves the quality of the pulp without losing the pulping yield. Concerning the effect of bleaching temperature, as observed from Figure 2, increasing the temperature from 30 to 45 °C, the pulp yield and the lignin content obviously decreased, and slowed down between 45 to 60 °C. Thus, 45 °C seems to be the optimum temperature because, at this temperature, important decreases in the pulp yield and in the lignin content were observed.

Table 4
Quadratic models fitted for yield and kappa number according to the studied factors of the pulping treatment (coefficients with uncoded data)

Terms	**Almond stems**		**Fig stems**	
	Yield (%)	Kappa	Yield (%)	Kappa
Constant	43.097	47.09	64.277	54.27
X_1	- 4.324	- 5.811	- 4.443	- 6.106
X_2	-1.105	- 1.236	- 0.834	- 1.961
X_3	-1.731	-0.877	- 1.856	- 5.313
$X_1 \times X_1$	0.750	- 1.537	0.494	- 2.18
$X_2 \times X_2$	-0.567	- 0.117	-0.798	0.51
$X_3 \times X_3$	1.315	0.460	1.382	1.72
$X_1 \times X_2$	-1.035	1.578	- 0.708	1.35
$X_1 \times X_3$	0.257	1.715	- 0.343	- 1.67
$X_2 \times X_3$	0.790	0.215	0.450	- 1.40
R-sq	97.09%	94.67%	98.89%	94.41%
R-sq(adj)	93.64%	85.09%	96.91%	84.35%
Predicted and experimental values of the responses under optimal conditions of pulping				
Optimized values (predicted values)	48.59	46.55	60.16	45.18
Experimental values*	49.35 ±0.35*	46.38 ± 0.12*	61.47 ± 0.38*	45.20± 0.12*

X_1: Temperature (°C); X_2: Time (min); X_3: Sodium hydroxide concentration (%); *Experimental values = mean values ± standard deviations

Table 5
Box-Behnken experimental design used for the bleaching process

Expt. N°	Factors				Responses			
					Almond stems		Fig stems	
	X_1	X_2	X_3	X_4	Yield (%)	Kappa	Yield (%)	Kappa
1	0	0	-1	1	60.15	06.12	76.44	07.04
2	-1	0	0	1	61.28	07.03	62.54	09.15
3	0	1	-1	0	72.46	09.29	85.37	14.45
4	-1	0	1	0	57.39	05.35	54.50	05.38

5	1	1	0	0	60.42	04.38	55.48	03.26
6	0	0	1	1	53.39	06.21	51.63	07.38
7	0	-1	0	1	61.17	07.44	58.39	10.65
8	0	0	0	0	56.28	06.09	74.47	07.30
9	-1	1	0	0	54.25	04.11	60.11	03.21
10	1	0	0	1	46.43	03.02	68.28	01.28
11	0	0	0	0	56.16	06.27	74.44	07.37
12	0	-1	1	0	70.41	05.25	84.43	06.64
13	0	1	1	0	39.58	07.48	53.54	10.31
14	-1	0	-1	0	60.29	07.50	92.48	09.05
15	1	-1	0	0	69.11	07.89	80.26	12.11
16	0	1	0	1	65.38	04.32	70.19	03.25
17	0	0	-1	-1	76.74	07.49	80.41	09.51
18	0	1	0	-1	56.47	05.77	60.68	08.33
19	-1	0	0	-1	56.28	08.14	78.29	12.58
20	1	0	-1	0	65.15	05.27	68.54	07.66
21	1	0	1	0	47.53	04.19	60.18	03.27
22	0	0	0	0	55.44	06.04	75.29	07.55
23	0	-1	-1	0	80.36	06.09	80.10	07.47
24	0	0	1	-1	55.41	05.22	68.06	05.29
25	0	-1	0	-1	78.53	06.11	85.53	09.18
26	-1	-1	0	0	92.93	09.26	91.81	14.07
27	1	0	0	-1	62.66	07.10	74.64	09.30

X_1: pH; X_2: Temperature (°C); X_3: Sodium hypochlorite concentration (%); X_4: Time (min)

With regard to the bleaching time, it can be concluded that whatever the starting material, the bleaching time affects the quality of the pulping. The more the bleaching time was increased, the larger amount of lignin could be removed and consequently the pulping yield decreased simultaneously.

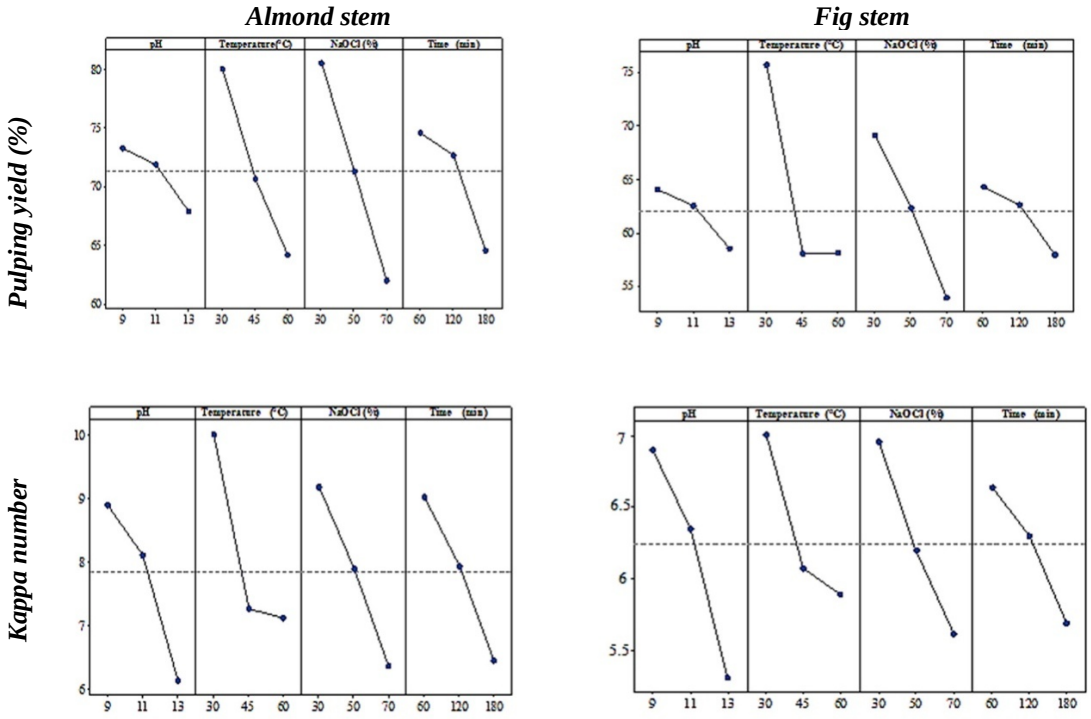

Figure 2: Main effects plot of bleaching parameters for pulping yield and kappa number of almond and fig stems

Finally, concerning the sodium hydrochlorite concentration, it was established that the sodium hypochlorite concentration plays an important role during the bleaching step. It can be noticed

that when the concentration increased from 30 to 70%, the pulping yield decreased from 70 to 50%. Lignin and cellulose are dissolved at different speeds during cooking and these rates are accelerated by increasing the sodium hypochlorite concentration.

The statistical analysis approach was applied again on the experimental results, using the obtained values of bleaching yield and kappa number, in order to accomplish the regression models and the evaluation is shown in Table 6. It can be noted that the obtained value of the determination coefficient (R^2 > 90% and R^2 (adj) > 79%) indicates again a good relation between the predicted and experimental values. The optimum conditions obtained by the Box-Behnken statistical design were determined to maximize the production of pure bleached cellulose. The results of the second-order polynomial model developed indicated that the optimal conditions for the bleaching process conditions are as follows: pH 12, temperature – 30 °C, NaClO concentration – 40% and time – 90 min. In order to evaluate the validity of the optimized conditions, experiments were performed to evaluate the experimental results *versus* predicted values of the output using the model equation. The experiments were carried out three times and the average values were presented in Table 6.

Table 6
Quadratic models fitted for yield and kappa number according to the studied factors in the case of bleaching process (coefficients with uncoded data)

Terms	Almond stems		Fig stems	
	Yield (%)	Kappa	Yield (%)	Kappa
Constant	55.96	6.133	74.73	7.41
X_1	-2.76	-0.795	-2.70	-1.38
X_2	-8.83	-0.557	-7.93	-1.44
X_3	-3.64	-0.672	-9.25	-1.41
X_4	-3.19	-0.474	-5.01	-1.29
$X_1 \times X_1$	1.24	-0.163	-2.39	-0.32
$X_2 \times X_2$	9.66	0.356	-0.04	1.25
$X_3 \times X_3$	1.64	0.115	-1.32	0.07
$X_4 \times X_4$	1.02	-0.072	-3.89	0.01
$X_1 \times X_2$	8.00	0.410	1.73	0.50
$X_1 \times X_3$	-3.68	0.268	7.40	-0.18
$X_1 \times X_4$	-5.31	-0.742	2.35	-1.15
$X_2 \times X_3$	-5.73	-0.242	-9.04	-0.83
$X_2 \times X_4$	6.57	-0.695	9.16	-1.64
$X_3 \times X_4$	-7.62	0.590	-3.11	1.14
R-sq	90.25%	90.00%	92.75%	90.14%
R-sq(adj)	79.88%	79.01%	84.29%	81.38%
Predicted and experimental values of the responses at optimal conditions of pulping treatment				
Optimized values (predicted	76.07	5.02	84.52	8.37
Experimental values*	77.79 ± 0.37*	6.65 ± 0.17*	85.26 ± 0.38*	9.78 ± 0.19*

X_1: pH; X_2: Temperature (°C); X_3: Sodium hypochlorite concentration (%); X_4: Time (min); *Experimental values = mean values ± standard deviations

Table 7
Characteristics of fibres extracted from almond and fig stems

Characteristics		**Values**	
		Almond stems	Fig stems
Morphological behaviour	Fibre length (mm)	0.518	0.516
	Fibre width (µm)	19.6	22.4
	Fine elements (%in length)	36.3	39.0
	Macrofibrils (% in length)	1.260	0.943
	Coarseness ($mg.m^{-1}$)	0.0469	0.0651
Crystallinity indexCI(%)	Raw material	41	46
	Cellulose	73	71
Degree of polymerization (DP)		1571	1563

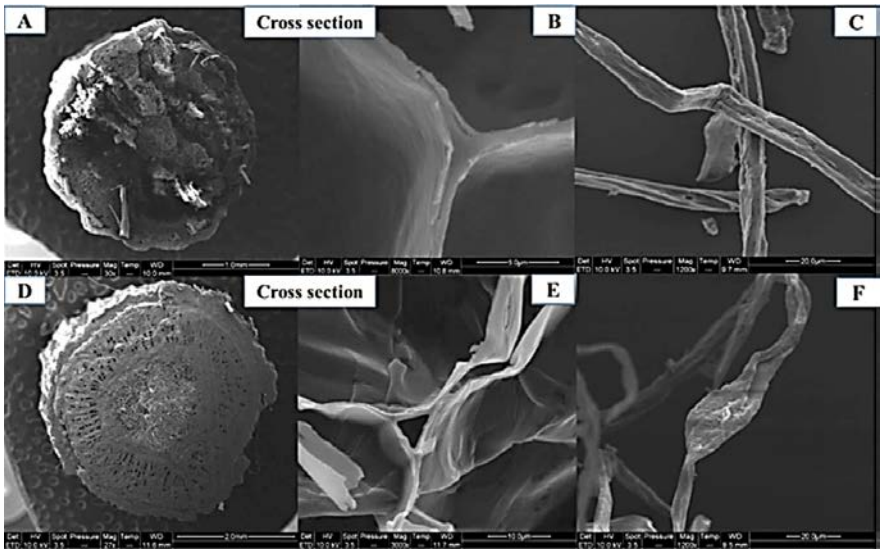

Figure 3: Scanning electron micrographs of (A and B) almond stem, (C) cellulosic fibre extracted from almond stems, (D and E) fig stem and (F) cellulosic fibre extracted from fig stems

The values obtained through confirmation experiments fall within 95% of the predicted values. This shows that the developed quadratic models are well suited. It is also evident that the optimal values are valid within the specified range of the process variables.

Overall, the delignification and bleaching conditions of the extracted fibres seem to be promising and interesting considering the low temperature and consumption of chemicals, as well as the reaction time. Moreover, the pulping yield and Kappa number achieved are close to those obtained from kraft pulping of softwood and hardwood, while the pulping yield is higher than that reported for annual plants and agricultural crops, generally around 35%.[25,26]

Morphological investigation

The morphological features of the stems were examined by cross-section analysis, using scanning electron microscopy and a Morfi device (Table 7). Figure 3 reveals complete separation of the cellulose fibres. This observation confirms the total removal of the non-cellulosic layer, comprising materials such as hemicelluloses, lignin and other impurities contained in the almond and fig stems. The obtained images (Fig. 3) exhibit that the almond and fig stems possess a well-distributed pore structure and high pore volume. The micrographs also reveal the presence of conducting vessels. The fig stem cells display a very thin wall, with the thickness in the order of 1 μm. The average lateral fibre dimensions and lateral fibre aggregate dimensions were estimated and the results are displayed in Table 7. It can be noticed that the fibres present the same morphological characteristics in terms of their length and diameter. The degree of polymerization (DP) of the cellulose was determined using the gel permeation chromatography (GPC) technique. As shown in Table 7, based on GPC analyses, the DP of the fibres extracted from the almond and fig stems were 1571 and 1563, respectively. The ensuing fibres from the two materials exhibit significant mechanical properties.

In addition, the X-ray powder diffraction (XRD) patterns were analysed. The diffraction patterns essentially show the low ordered amorphous and highly ordered crystalline regions in the isolated cellulose samples. The relative quantities of these two regions determine the crystallinity of the cellulose. The celluloses extracted from almond and fig stems showed four diffraction peaks at 2θ = 14.9°, 16.1°, 22.2° and 34.8°, characteristic of cellulose crystal I.[27] The removal of lignin and hemicelluloses from the almond and fig stems was indicated by the shoulder peak at 16.1° and a weak peak at 34.8°. Moreover, a new diffraction peak at around 2θ = 19.7° was assigned to the less ordered or amorphous region of the cellulose chains.[28] The crystallinity indices for both materials are presented in Table 7. The estimated crystallinity index was 73 and 71% for the celluloses extracted from almond and fig stems, respectively. It can be noticed that the crystallinity index of the cellulose

fibres from almond stems was higher than that of the fibres from fig stems.

CONCLUSION

In this study, the optimization of pulping conditions for maximizing the pulping yield and minimizing the kappa number of the pulp were performed using the Box-Behnken experimental design. The results indicated that the effect of the treatment variables, including temperature, contact time and soda concentration, was statistically significant for increasing the yield and decreasing the kappa number. The temperature plays a predomint effect. The soda concentration rank the second and the contact time seemed to have the least effect on the soda-anthraquinone process. The optimal process conditions obtained to maximize the production of unbleached fibres were as follows: temperature – 120 °C, time – 70 min and NaOH concentration – 9%; and temperature – 135 °C, time – 75 min and NaOH concentration – 15%, for almond and fig stems, respectively. Following the soda-anthraquinone treatment, the optimization of the bleaching conditions was studied and the optimum conditions were found to be the following: pH 12, temperature – 30 °C, NaClO concentration – 40% and time – 90 min. The obtained fibres were characterized by several methods and it was proven that both types of biomass can be considered as potential sources of cellulose for the production of cellulose derivatives and lignocellulosic fibres for fibre-reinforced composites, as well as for papermaking applications.

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