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DETERMINATION OF SUGAR CONTENT IN *PINUS PINASTER* AND ITS CORRESPONDING HOLLOCELLULOSE, CELLULOSE AND KRAFT PULPS

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This paper deals with the study of the polysaccharide complex from Portuguese *Pinus pinaster*, as well as from its main components, i.e., holocellulose, cellulose and pulps obtained using different cooking conditions. Nordic *Pinus sylvestris* was also studied in order to compare it with Portuguese pine. Two methods were used: one consisted of the hydrolysis of polysaccharides with trifluoroacetic acid (TFA) and quantification of the amount of each sugar using High Performance Liquid Chromatography (HPLC). The latter approach used here consisted of the hydrolysis of the samples under scrutiny by sulphuric acid and converting it into alditols, which were then esterified before injecting to Gas Chromatography coupled with Mass Spectrometry. Myo-inositol was used as an internal standard. The results obtained by the two methods showed that the TFA procedure prevents monosaccharide degradation, comparatively with the sulphuric acid one. In fact, the overall sugar yield was found systematically higher than 93% for TFA hydrolysis, whereas that corresponding to H_2SO_4 treatment never exceeded 77%. The sugar composition of both pines was found very similar and the sulfidity level (from 10 to 55%) did not affect the pulp sugar composition. The glucose/mannose and xylose/arabinose ratios were found to be around 3.5 for both Portuguese and Nordic pines. Finally, kraft pulping of *Pinus pinaster* was found to affect drastically galactose, mannose and arabinose.

Key words: polysaccharide, *Pinus pinaster*, sugar, hollocellulose, kraft pulp.

INTRODUCTION

The study of sugar composition from wood and pulps has been for a long time a matter of discussion, in terms of results' viability and reproducibility.[1] In fact, these analyses are usually carried out in two steps, namely: (i) hydrolysis of the carbohydrate complex, and (ii) quantification of the ensuing hydrolysate. The most commonly used conditions of wood and pulps hydrolysis consisted of a two-step technique, i.e.

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treatment of the woody material at room temperature, by a solution of 72% sulphuric acid, followed by the dilution of the acid concentration to 5% and heating the resulting mixture for 1 hour at 120°. The sugars are then reduced to the corresponding alditols, derivatised by esterification with acetic anhydride and quantified by Gas-Liquid Chromatography.[2] Recently, this standardized[3] method has been modified by Cao *et al.*,[4] who proposed *N*-methyl imidazole as a new catalyst for the esterification reaction. These drastic conditions induced degradation of a given amount of sugars.

Fengel and Wegener[5] have proposed milder hydrolysis conditions to quantify sugars of woods and pulps. Thus, a softer acid, i.e. trifluoroacetic acid (TFA) and lower temperatures (70–100°) were used. Their method has several advantages, namely: (i) it is less time consuming, (ii) the reduction and derivatisation steps are avoided, (iii) the TFA is more easily removed, and (iv) the total sugars yield is higher, which suggests less carbohydrate degradation. Moreover, quantification by High Performance Liquid Chromatography gave simple chromatograms, and sugar separation is excellent.

The main objective of this paper is to find the optimal conditions of the TFA method, as applied to Portuguese *Pinus pinaster* itself and also to different pulps obtained from its cooking with different conditions. In fact, our laboratory has been involved in a large program of research investigating this softwood.[6-9] The main objectives of these works refer to the structure of residual and dissolved lignin from kraft pulps of *Pinus pinaster*,[6,7] the evaluation of Portuguese pine fibre quality[8] and the comparative study of the chemical composition of different pines.[9]

RESULTS AND DISCUSSION

Before starting the study of sugar composition of *Pinus pinaster* and of its derivatives, i.e. the corresponding holocellulose and kraft pulps, the calibration curves for cellobiose, glucose, xylose, galactose, arabinose and mannose were established, using commercial products. A typical chromatogram from this set of experiments is presented in Figure 1. Six linear equations were obtained with excellent square of the correlation coefficients and an intercept very close to zero, as summarised in Table 1.

TABLE 1

Calibration equations for each sugar

Sugar	Retention time, min	Equation (y = ax + b)		R^2
		$a \cdot 10^{-5}$	b	
Cellobiose	17.0	1.23	1.08	0.9996
Glucose	20.4	1.26	0.73	0.9998
Xylose	21.9	1.27	1.72	0.9994
Galactose	23.9	2.25	1.05	0.9998
Arabinose	25.8	1.32	1.97	0.9998
Mannose	27.5	1.26	3.73	0.9994

y is the sugar quantity in mg and x the peak area in arbitrary units.

Then, the hydrolysis conditions of Portuguese pine and of its derivatives were optimised, considering the minimum sugar degradation as the main criteria. Figure 2 shows a typical chromatogram corresponding to the sugar detected from *Pinus pinaster* hydrolysis. From this chromatogram, it could be seen that the peaks are symmetrical, well-defined and sharp, and the retention times of the corresponding sugars are very close to those obtained from the standard ones. Moreover, except the peaks corresponding to the investigated sugars, no other peaks were ever detected. In addition to these precious indications, the hydrolysate was submitted to elemental analysis and FTIR spectroscopy, which showed the absence of fluorine. This indicates that the esterification reaction between TFA and the hydroxyl groups of sugars could be neglected.

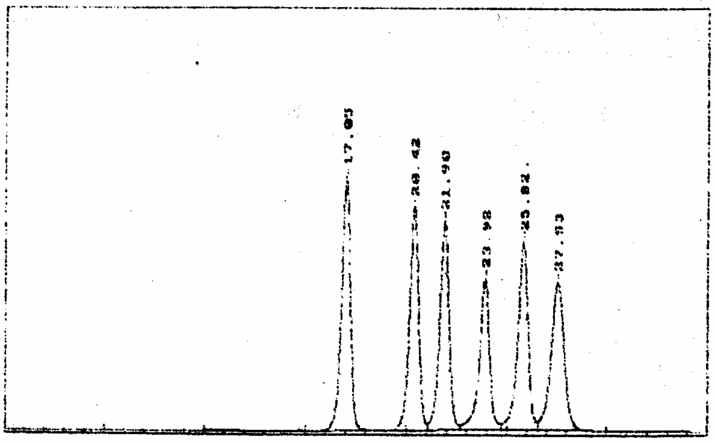

Fig. 1 – Typical chromatogram dealing with calibration curves establishment.

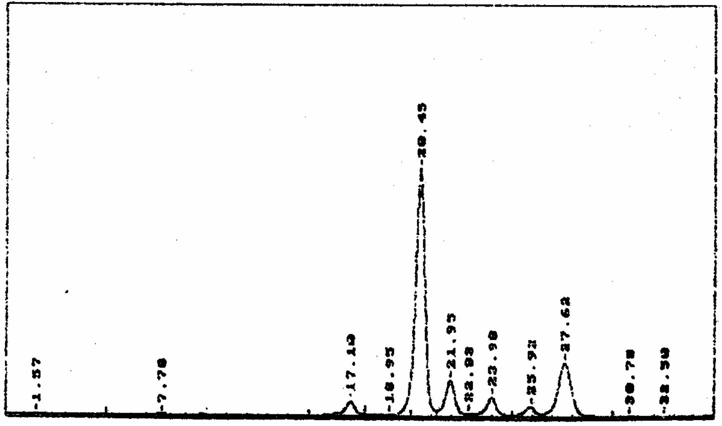

Fig. 2 – Typical chromatogram corresponding to the analysis of sugar composition of *Pinus pinaster*.

An example of the optimisation approach, as applied to unbleached kraft pulps from *Pinus pinaster*, is given in Figure 3, which presents the evolution of the individual sugar content, as a function of the hydrolysis conditions, as summarised in Table 2. From this Figure, one could appreciate the significant effect of the hydrolysis conditions on the total sugars yield.

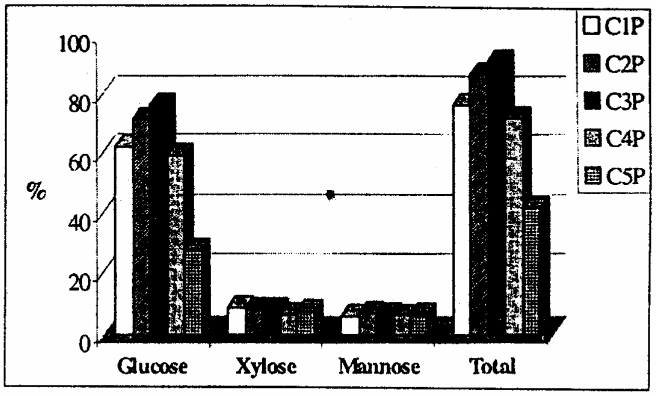

Fig. 3 – Evolution of sugar composition of unbleached kraft pulps from *Pinus pinaster*, as a function of the hydrolysis reaction conditions.

TABLE 2

Optimisation of the TFA hydrolysis of different materials from *Pinus pinaster*

		Step					
		First		Second		Third	
Material	Conditions	[TFA], %	Time, min.	[TFA], %	Time, min.	[TFA], %	Time, min.
Wood	C1W	100	20	85	15	39	60
	C2W	100	20	85	15	39	30
	C3W	100	30	80	15	20	60
Pulps	C1P	100	20	85	15	39	60
	C2P	100	20	85	15	39	30
	C3P	100	20	80	15	20	60
	C4P[a]	100	20	80	15	20	60
	C5P[b]	100	20	80	15	20	60
Holocellulose	C1H	100	20	85	15	39	60
	C2H	100	20	85	15	39	30
	C3H	100	20	80	15	20	60

[a] Oil bath, [b] water bath; the rest of the experiments were carried out with an electric heater.

The optimum conditions which should be applied to pulp samples are those reported in the C3 experiment, since these conditions allowed to reach a total yield as high as 95%. Moreover, the optimal conditions are material dependent. In fact, the reaction time of the first hydrolysis step of wood is much longer than that for pulps and holocellulose. This is due to the reduced accessibility of polysaccharide in wood samples, comparatively with that of pulps and holocellulose. Table 3 shows the optimum conditions established for each raw material.

TABLE 3

Optimal TFA hydrolysis conditions of different materials from *Pinus pinaster*

	Hydrolysis step					
	First		Second		Third	
Material	[TFA], %	Time, min.	[TFA], %	Time, min.	[TFA], %	Time, min.
Wood	100	30	80	15	20	60
Pulps	100	20	80	15	20	60
Holocellulose	100	20	80	15	20	60
Cellulose	100	20	80	15	20	60

TABLE 4

Sugar composition of different materials studied, as determined by the TFA method and HPLC quantification (values in parentheses resulted from the correction to 100% of yield)

	Sugar content, % (w/w with respect to O. D. material)					
Material	Glucose	Xylose	Galactose	Arabinose	Mannose	Total yield
Pinus pinaster (PP)	63.8 (65.1)	8.3 (8.5)	5.2 (5.3)	2.3 (2.3)	18.4 (18.8)	98.0 (100)
Pinus sylvestris	61.4 (65.9)	7.9 (8.5)	4.9 (5.3)	2.4 (2.6)	16.6 (17.8)	93.2 (100)
Holocellulose from *PP*	61.9 (66.2)	9.9 (10.6)	4.0 (4.3)	2.8 (3.0)	14.9 (15.9)	93.5 (100)
Pulps prepared from *PP*, with a sulfidity of						
10 (Pulp 1)	75.9 (81.2)	8.7 (9.3)	1.1 (1.2)	1.1 (1.2)	6.7 (7.2)	93.5 (100)
25 (Pulp 2)	77.5 (81.4)	8.9 (9.3)	0.9 (0.9)	1.0 (1.1)	6.9 (7.2)	95.2 (100)
40 (Pulp 3)	77.7 (82.0)	8.5 (9.0)	0.8 (0.8)	1.0 (1.1)	6.7 (7.1)	94.7 (100)
55 (Pulp 4)	77.9 (82.6)	8.2 (8.7)	0.8 (0.8)	1.0 (1.1)	6.4 (6.8)	94.3 (100)

The sugar composition of *Pinus pinaster* and of its derivatives, as determined in optimum conditions, is presented in Table 4, which gives the values obtained and those corrected to 100% of yield. From these data, the following remarks could be drawn:

(i) as expected, the main sugars detected are glucose, xylose, galactose, arabinose and mannose,

(ii) the total yield of sugars could be considered very good, since it was systematically higher that 93%,

(iii) the quantities of each sugar in the wood sample for both pines are very close to each other,

(iv) the ratio between glucose and mannose is very similar for both pines, i.e. 3.5 and 3.7 for *Pinus pinaster* and *Pinus sylvestris*, respectively, which is in good agreement with the literature,[10]

(v) the xylose/arabinose ratio was found to be 3.6 and 3.3 for Portuguese and Nordic pines, respectively, which is much lower than the values reported in the literature for softwoods,[11, 12]

(vi) the sugar composition of holocellulose is very close to that of the initial wood (*Pinus pinaster*), which indicates that the isolation of holocelluloses is quite selective. In fact, the yield of holocellulose from Portuguese pine was about 68.4%, with a negligible amount of residual lignin.[13] Nevertheless, the exception which should be mentioned concerns the content of arabinose and xylose, which was higher for holocellulose in comparison with initial wood. This could result from the more drastic initial step of wood hydrolysis (treatment with concentrated TFA for 30 min, instead of 20 min) and/or the loss of some galactoglucomannans during the isolation of holocellulose,[11,14]

(vii) during cooking, galactose, arabinose and mannose suffer considerably, which corroborates very well with literature data,[11, 14]

(viii) the carbohydrate complex composition was not significantly affected by the increase of the sodium sulfide concentration. This was already observed for the alkaline cooking of other woods,[12-15]

(ix) the glucose-to-mannose ratio in the unbleached kraft pulps from *Pinus pinaster* was around 11.6, which is slightly higher than that reported in the literature for kraft pulps from other softwoods,[12] i.e. 8.7, and, finally

(x) the xylose-to-arabinose ratio was around 8, which is lower than that reported in the literature (11.4).

The second set of experiments made in this study concerns the use of alditols method to quantify the sugar composition of wood and of its derivatives, as summarised in Table 5.

The first remark concerning this method refers to the fact that the total yield of sugars is quite low, comparatively with that observed with the TFA method. In fact, only less than 80% of initial sugars were recovered, which corroborates quite well with bibliographic data.[4] Furthermore, glucose and xylose were found to be very stable in sulphuric acidic solution, once conjugated with high temperature (120º), whereas the other sugars showed a substantial degradation under the same treatment, as evidenced by the values reported in Table 5, for galactose, arabinose and mannose. Indeed, the weak stability of arabinose under acidic conditions was already observed in the literature.[11] Moreover, the ratio between glucose and mannose was found to be very different, comparatively with that observed with the TFA hydrolysis method, i.e. 6.5 and 5.9 instead of 3.5 and 3.7, for Portuguese and, respectively, Nordic pine. The xylose/arabinose ratio was found to be 9.4 and 6 for both pines studied here, which is closer to the values reported in literature.[11] For pulps, a ratio of 45 between glucose and mannose was found, which is absurd. The low sugar yield, the total disappearance of arabinose and galactose and the strong degradation of mannose make the alditol method contestable.

TABLE 5

Sugar composition of *Pinus pinaster* and of its derivatives, as determined by alditols method and quantified by GC-MS (values in parentheses resulted from the correction to 100% of yield)

	Sugar content, % (w/w with respect to O.D. material)					
	Glucose	Xylose	Galactose	Arabinose	Mannose	Total yield
Pinus pinaster	58.7	7.5	0.1	0.8	9.0	76.1
	(77.1)	(9.9)	(0.1)	(1.1)	(11.8)	(100)
Pinus sylvestris	58.4	7.2	0.0	1.2	10.1	76.9
	(75.9)	(9.4)	(0.0)	(1.6)	(13.1)	(100)
Pulps 1	61.6	5.2	0.0	0.0	1.3	68.1
Pulps 4	(90.5)	(7.6)	(0.0)	(0.0)	(1.9)	(100)
	61.2	5.4	0.1	0.0	1.4	68.1
	(89.9)	(7.9)	(0.1)	(0.0)	(2.1)	(100)

CONCLUSIONS

This work showed clearly that even standard methods should be optimised before being applied to species never investigated in the given field. Thus, this contribution demonstrated that the trifluoroacetic acid method, completed by High Performance Liquid Chromatography quantification is suitable for sugar composition of *Pinus pinaster* and of its derivatives. The method, based on sulphuric acid hydrolysis, followed by conversion of sugars to alditols, is less selective, since it induces higher degradation of monosaccharides. Moreover, kraft cooking of *Pinus pinaster* was found to degrade essentially galactose, arabinose and mannose. The carbohydrate composition was not affected by increasing the sulfidity level during kraft cooking.

EXPERIMENTAL

Polysaccharides were hydrolysed according to the TFA method,[5] as optimised for our study. In fact, the time of each step, as well as the concentration of TFA, were varied, and the optimal conditions selected, as discussed above. The second method of sugar determination applied in our study was carried out following the modified Tappi standard T-249 cm-85, as reported by Cao *et al.*[4]

The wood samples investigated here were commercial wood chips supplied by pulp mills. Holocellulose was determined according to the well-known peracetic method.[16] The pulps prepared here were obtained by standard kraft cooking of wood ships. The effective alkali was of 18.4% and the different sulfidity levels were 10, 25, 40 and 55%, respectively, while the time-temperature program was the following: heating from room temperature to 170° for 120 minutes and maintaining at this isotherm for 113 minutes. The kappa numbers obtained were 75, 48, 36 and 31 for pulps 1 to 4, respectively.

High Performance Liquid Chromatography (HPLC) measurements were carried out using a Perkin Elmer 250 chromatograph equipped with a commercial column ("Polysphere OH-PB" from Merck Co.), and a refractive index detector (HP 1074A, from Hewlett Packard). This column is filled by an organic support and it is based on the cationic ion-exchange principle of separation (Pb^{++}). A flow rate of 0.4 mL per minute of bi-distilled, degassed water and a temperature of 80°, were used. Before the injection of our samples, calibration curves of cellobiose, D-glucose, L-arabinose, D-galactose, D-xylose and D-mannose were obtained using high purity commercial sugars from Merck Co.

The GC-MS used here was a Fison GC-8000 series with an MS Trio 1000, equipped with a commercial silica fused capillary column (DB-225). The separation conditions were the following: injection port temperature was 250° and the oven maintained at an isotherm of 220°. The potential of ionisation of the source was 70 eV.

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