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Keywords

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^{13}C NMR

Summary

Extractive-free cork from *Quercus suber* L. was submitted to organosolv fractionation and the effects of different process variables, such as ethanol/water ratio, temperature, time and the presence of acidic or alkaline catalysts, were studied. The variation of the relative proportions of extracted components, as a function of the processing conditions, could thus be established. Whereas the addition of 0.1 M acetic acid only increased the yield of extracted materials from about 15 to 23 %, the use of sodium hydroxide, at the same concentration, produced a jump to 76 %. In the case of the alkaline organosolv fractionation, an increase in process temperature, time and catalyst concentration led to an increase in the extraction yield, although in some cases this increase did not follow a sustained trend, as in the case of reaction time. Increasing the ethanol/water ratio led to a higher selectivity in favour of suberin extraction. Residual cork from different organosolv processes was characterised by FTIR and ^{13}C solid-state NMR. The latter technique provided some valuable information about both process selectivity and cork morphology, particularly with respect to the positioning of suberin macromolecules in the cell wall.

Introduction

Cork of *Quercus suber* L. is composed of suberin, a predominantly aliphatic biopolyester, the main cork component contributing to about 40 % of its dry weight, and lignin, polysaccharides and extractives in equivalent amounts (Cordeiro *et al*. 1998 a). Most of the studies which have been carried out up to now on the quantification and characterisation of cork components involved a range of chemical treatments (Pereira 1988) which were rather severe and could therefore be accompanied by degradation processes. As a consequence, little information is available on details related to the association among different components within the cork morphology. When milder approaches were applied (Marques *et al*. 1996), the amounts of extracted components were very small and their ensuing characterisation left some uncertainty about their relevance with respect to the main constituents.

Within a general research programme dealing with cork chemistry (Pascoal Neto *et al*. 1995; Cordeiro *et al*. 1995; Pascoal Neto *et al*. 1996; Gil *et al.*, 1997; Cordeiro *et al*. 1996, 1998 a, b; Lopes *et al*. 1998) and with the possible exploitation of its components (Cordeiro *et al*. 1998 a; Cordeiro *et al*. 1999, 2000), we applied the organosolv fractionation techniques to cork in order to achieve a mild and efficient fractionation.

The organosolv technique involves the treatment of lignocellulosic substrates with organic solvent-water media at high temperatures (140–180 °C) in the presence or absence of a catalyst. This type of process has been previously applied extensively to the fractionation of wood components (Lora and Aziz 1985; Pascoal Neto *et al.* 1994; Evtuguin *et al.* 1999). It has been performed with a great variety of solvents using acid (mineral, organic or Lewis acids) or alkaline catalysts (sodium hydroxide or ammonia). The fractions obtained have different characteristics depending on the specific process conditions. In this work, we have fractionated the cork components by an organosolv treatment based on the use of ethanol/water media and characterised the residual cork by FTIR and ^{13}C-solid-state NMR.

Materials and Methods

Cork powder samples were obtained by grinding high-quality reproduction cork kindly supplied by the Champcork Company of Portugal. The composition of this sample was determined by classical methods (Pereira 1988) and found to be: 47 % of suberin, 18 % of lignin, 16 % of polysaccharides and 19 % of extractives (o. d. cork). This powder was extracted sequentially with dichloromethane, ethanol and water in a soxhlet apparatus (8 h for each solvent). The extractive-free cork powder was dried to constant weight and then treated with the solvent mixture in a 4842 PARR stirred reactor working under pressure. Processing conditions were as follows: i) solvent mixture: (ethanol:water, v:v) 0:100, 20:80, 40:60, 50:50, 80:20, 100:0; ii) solvent/cork ratio: 20/1 and 40/1 (l/kg); iii) catalyst: none, 0.1M acetic acid; 0.1M H_2SO_4; 0.05, 0.1, 0.3 and 0.5M sodium hydroxide; iv) temperature: 110, 120, 140 and 160 °C; v) time: 1, 2, 3 and 4 hours at constant temperature. After cooking, the reactor was cooled and the solid fraction separated from the black liquor by filtration. The solid residue was then washed with water, dichloromethane and diethyl ether and finally dried to constant weight.

The FTIR spectra were taken with a Mattson 7000 spectrophotometer using the standard KBr pellet technique. Solid-state ^{13}C NMR spectra were recorded on a Bruker MSL-400 spectro-

meter at 100.6 MHz, at room temperature, with rotors spinning at 5.9 KHz. The CP-MAS spectra were recorded with 3 s recycle delay and 1.5 ms contact time.

Results and Discussion

Effect of the addition of acid and basic catalysts

Table 1 shows the results of organosolv treatment of cork with ethanol/water media in terms of the percentage of extracted material. The uncatalysed treatment of cork yielded 14.7 % of dissolved substances. This relatively low yield, compared with those obtained with woody materials (Pascoal Neto *et al*. 1994), was attributed to the fact that closed cells and the presence of suberin in the cell walls limited the diffusion of the liquid mixture inside the cork structure. The addition of 0.1 M acetic acid increased the yield to only 23.0 % for the same 4 h and to 27.2 % for 12 h processing. This modest increase can be explained by the resistance of the cork structure to the attack by (weak) acidic media in contrast to alkaline ones. In fact, the ester bonds in suberin can be readily hydrolysed by bases, as clearly shown by the substantial yield increase to 76.3 % when the same concentration of NaOH was added to the liquid phase. These results suggest that i) suberin is present in cork as a "coating" enveloping the other components and/or ii) suberin is chemically associated with the other components, making it impossible to separate them efficiently without the preliminary hydrolysis of the ester linkages. This conclusion is corroborated by the fact that

Table 1. Processing conditions and results of organosolv fractionations applied to cork of *Quercus suber* L

Catalyst	Ethanol/water	Temperature (°C)	Catalyst (M) Concentration	Time (h)	% dissolved material (extracted cork)
Without	50/50	160	–	4	14.7
Acetic acid	50/50	160	0.1	4	23.0
Sodium Hydroxide	0/100	160	0.1	4	39.4
	20/80				39.9
	40/60				45.6
	50/50				76.3
	80/20				72.0
	100/0				69.9
	50/50	110	0.1	4	30.1
		120			41.9
		140			64.6
		160			76.3
	50/50	160	0.05	4	42.6
			0.1		76.3
			0.3		85.0
			0.5		84.4
	50/50	160	0.1	1	35.4
				2	37.3
				3	46.3
				4	76.3

Table 2. FTIR assignments for cork components

Wavenumber (cm^{-1})		Assignment
3414	OH stretch	hemicellulose, cellulose, lignin, suberin
2937	CH aliph. stretch	suberin, hemicellulose cellulose, lignin
2850		suberin, hemicellulose cellulose, lignin
1745	C=O stretch	suberin, hemicellulose cellulose, lignin
1636	C=C stretch	suberin
1603		suberin, lignin
1513		suberin, lignin
1468	CH assym stretch	suberin, hemicellulose, cellulose, lignin
1384	CH sym stretch	suberin, hemicellulose, cellulose, lignin
1270	CO stretch	suberin, hemicellulose, cellulose, lignin
1164	CO assym. stretch	suberin, hemicellulose, cellulose, lignin
1107	CH, CO deform	hemicellulose, cellulose, lignin
1032		hemicellulose, cellulose, lignin

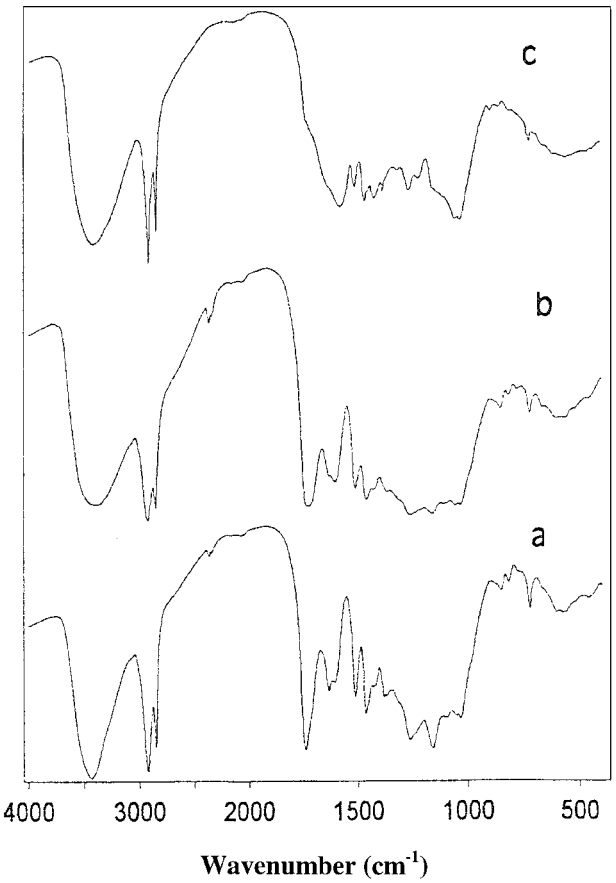

Fig. 1. FTIR spectra of residual cork form (a) uncatalyzed organosolv treatment, (b) in the presence of 0.1 M acetic acid and (c) with 0.1 M sodium hydroxide.

the low percentages of extracted materials with neutral or acidic treatments were composed essentially of saccharidic structures (Cordeiro 1998).

Figure 1 shows the FTIR spectra of residual cork from different processing conditions. Table 2 summarises the assignments of the corresponding bands, based on previous work (Pascoal Neto *et al.* 1995; Marques *et al.* 1996).

The spectrum of initial cork (not shown) and that of the residue following the uncatalysed extraction (Fig. 1 a) were very similar. They were characterised by an O-H stretch band (ca. 3415 cm^{-1}) and a dominant CH band, with two peaks (ca. 2935-2850 cm^{-1}) corresponding to the aliphatic moieties in lignin and carbohydrates, but mostly in suberin components (Table 2). The intense C = O stretching band at 1745 cm^{-1} is characteristic of aliphatic esters in suberin. The 1635-1515 cm^{-1} region corresponded to an aromatic C = C stretch, from aromatic suberin, but mainly from lignin components. The bands at 1468 cm^{-1} and 1384 cm^{-1} reflected C-H symmetric and asymmetric deformations, respectively. The region 1270-1032 cm^{-1} showed C-O stretch and deformation bands in cellulose, hemicelluloses and lignin, although in this region, suberin can also contribute to the absorption with the C-O stretching from its ester groups (Table 2).

In the FTIR spectrum of residual cork obtained in the presence of acetic acid (Fig. 1 b), small changes were observed only at 1745 cm^{-1} and in the bands corresponding to C-O stretch (1270 and 1164 cm^{-1}). After the alkaline treatment, the FTIR spectrum (Fig. 1 c) showed more significant differences, since the carbonyl band was reduced to a small shoulder at 1745 cm^{-1}, mainly from suberin. The presence of unremoved suberin was confirmed by the 2937 and 2850 cm^{-1} bands arising from aliphatic sequences. The increase in the bands at 1107 and 1032 cm^{-1}, relative to those at 1270 and 1164 cm^{-1}, suggested that lignin had been more largely removed in comparison to carbohydrate components.

Table 3. ^{13}C NMR assignments for cork components

Chemical shift (ppm)	Assignment
21	CH_3-COO-, hemicellulose
30	-(CH_2)n-, suberin
33	-(CH_2)n-, suberin
56	Ar-OCH_3, lignin, -OCH_3, hemicellulose
61–62	Cγ-OH, Cβ-OAr, lignin
	C6, cellulose
64	C6, carbohydrate attached to suberin
72	C2, C3, C5, cellulose, hemicellulose
	C2, C3, C5, carbohydrate attached to suberin
75	C2, C3, C5, cellulose, hemicellulose
	Cγ-OR, Cβ-OR, lignin
82	C4, carbohydrate attached to suberin
	C4, cellulose
89	C4, cellulose
	Cβ-OR, Cα-OR, lignin
105	C1, carbohydrate attached to suberin
	C1, carbohydrates
	C1, cellulose
	-CH=CH-, suberin
	G2, S2, S6, lignin
114	G5, lignin
	-CH-, aliphatic and aromatic, suberin
122–126	G6, Cβ, lignin
130	Quaternary C, aromatic, suberin
	Cα, lignin
148	G4, S4, lignin
151–152	Quaternary C, aromatic, suberin
	G3, S3, S5, lignin
173	-COO-, suberin
	CH3-COO-, hemicellulose

The above conclusions were confirmed by the ^{13}C NMR spectra of the same residues, as shown in Figure 2 (Table 3 gives the corresponding assignments). The analysis of these spectra was conducted on the basis of previous work reported for wood and other plant systems (Zlotnik-Mazoni and Stark 1988; Garbow *et al.* 1989; Stark and Garbow 1992). The most intense peak at 30 and 33 ppm in the spectrum of the initial cork (Fig. 2 a) corresponded to aliphatic CH_2 groups in suberin. The signal at 56 ppm resulted mainly from lignin, but hemicellulose -OCH_3 groups also contributed in small amounts to this signal. The presence of small amounts of hemicelluloses was confirmed by the signal at 21 ppm. The peak at 56 ppm was used to monitor the extraction of lignin. The peaks at 61–105 ppm arose from overlapping signals of carbohydrates and lignin aliphatic carbons. The resonances in the 105–148 ppm region were attributed to

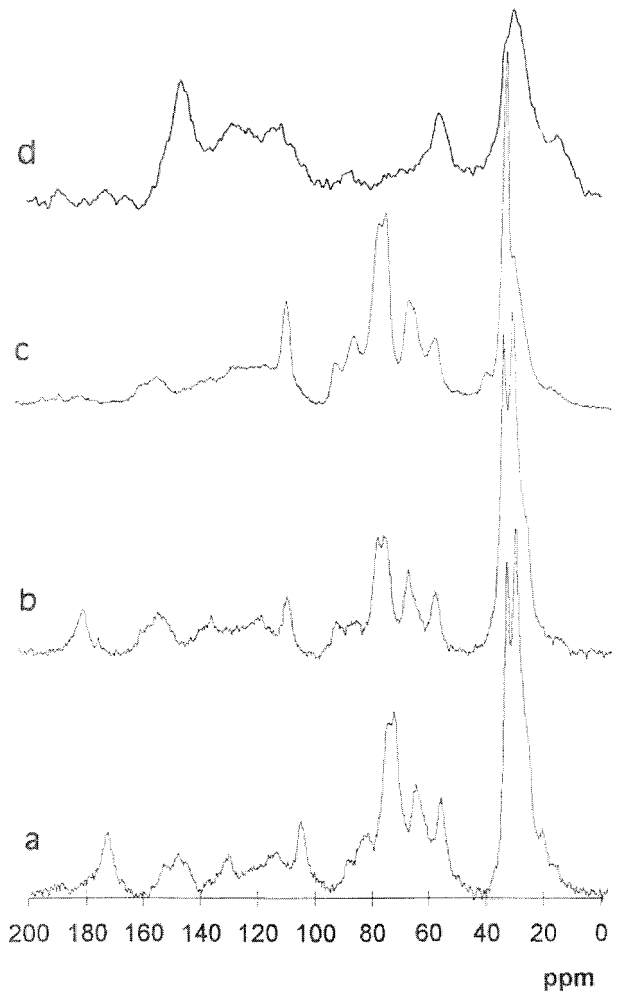

Fig. 2. ^{13}C NMR spectra of residual cork from (a) uncatalyzed organosolv treatment, (b) in the presence of 0.1 M acetic acid, (c) with 0.1 M sodium hydroxide and (d) with 0.1 M sulphuric acid.

unsaturated and aromatic carbons from lignin and/or suberin and the peak at 151–152 ppm to quaternary ring carbons. Finally, the carbonyl signal at 173 ppm confirmed the presence of acetyl groups from suberin and hemicelluloses.

The uncatalysed process gave a residue whose NMR spectrum was very similar to that of the untreated cork (Fig. 2 a), whereas the addition of acetic acid gave rise to significant differences (Fig. 2 b). The peak at 21 ppm disappeared, suggesting the thorough extraction of the acetylated hemicellulose component. The relative decrease in the intensity of the 72 and 82 ppm peaks indicated the partial extraction of polysaccharides (cellulose and hemicelluloses). The comparatively modest extraction of lignin was confirmed by the small decrease in the 56 and 151–152 ppm peaks. Finally, the characteristic peaks of suberin remained unperturbed. These observations confirmed that weak acidic conditions are not favourable to suberin extraction, but work better to remove hemicellulose, cellulose and lignin components (Pereira 1988).

In basic conditions, the changes in the NMR spectrum of the residue (Fig. 2 c) were more drastic, since the peak at 30 ppm showed a large decrease, the peak at 173 ppm disappeared and the peaks at 56 and 114–152 ppm decreased in comparison with those in the 64–105 ppm region. These results corroborated the conclusions drawn from the FTIR spectra, as indeed expected from the well-known fact that a basic medium favours the predominant extraction of suberin and lignin components (Pereira 1988).

A relevant aspect of this part of our study was the permanence of polysaccharide peaks as well as methylenes resonances at 33 ppm in residual cork, even with high alkaline concentrations (0.5 M NaOH) (Fig. 7). This suggests that these methylene groups were linked to the carbohydrate/lignin matrix by bonds resistant to that particular medium, *i. e.*, probably ether bonds. This also suggests that the other methylenes groups resonating at 30 ppm were part of COO ester moieties (173 ppm), and could thus be readily removed by alkaline hydrolysis or base-catalysed transesterification with ethanol. This result seems consistent with previous work on cork molecular dynamics (Gil *et al*. 1997; Lopes *et al* 2000) in which it was proposed that the less mobile methylene groups in suberin aliphatic chains, resonating at 33 ppm, were situated near the linkage to carbohydrate/lignin matrix, probably involving carbohydrate C_6 and C_β-OR and/or C_α-OR lignin groups. The more mobile CH_2 groups, resonating at 30 ppm, were those present in the same suberin chains, but far from the interpolymer junctions.

With the aim of increasing the extent of extraction in acidic conditions, a strong mineral acid was tried in conjunction with the ethanol/water mixture in the same conditions employed for the experiments with NaOH. When 0.1M sulphuric acid was added as a catalyst, the yield of solubilised materials reached 76.0 %. The FTIR spectrum of the residue showed features which clearly indicated that a substantial proportion of lignin remained unextracted. Conversely, most of the ester moieties were removed by this treatment.

The solid state ^{13}C NMR spectrum (Fig. 2 d) of this residue, compared with that of initial cork, displayed the following differences: i) a significant decrease in the aliphatic CH_2 signal at 30 and the disappearance of the 33 ppm peak, resulting from suberin extraction; ii) whereas the signal at 55 ppm, corresponding to methoxy carbons, was still present, the peaks at 64, 72 and 82 ppm disappeared, indicating that lignin had not been extracted extensively (as also shown by the FTIR spectrum), but that polysaccharides had been removed; iii) the vanishing of the signal at 172 ppm, corresponding mostly to COO groups in suberin; iv) the set of peaks between 110 and 160 ppm, corresponding to lignin aromatic groups, preserved relatively intense signals, particularly at 145 ppm, corroborating the evidence related to the presence of lignin in this residue.

Although the use of a strong acid provided a much higher extraction efficiency than weak ones, we also found that the severity of this treatment was excessive in terms of suberin degradation (Cordeiro *et al*. 2000). Since our main objective beyond these extractions was the possible exploitation of suberin as a source of polymeric materials (Cordeiro *et al*. 1997, 1999), it was decided to pursue this

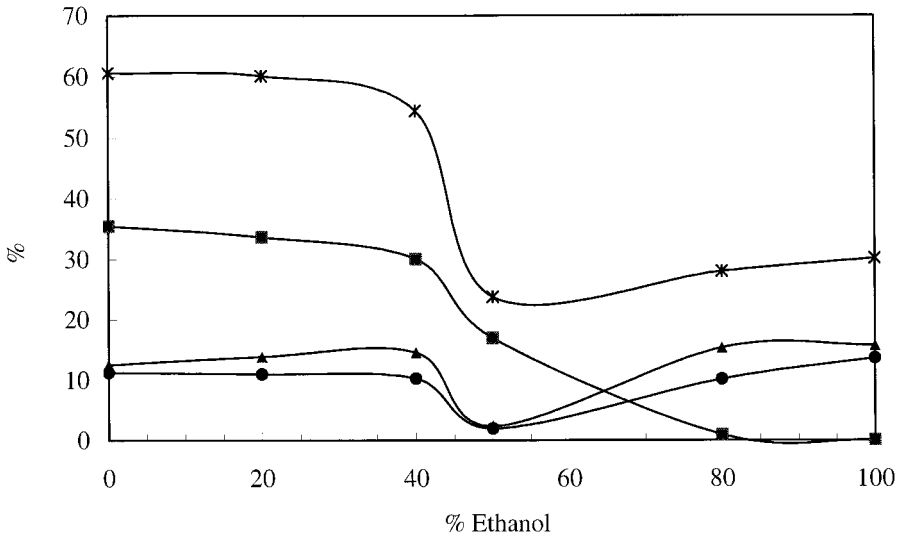

Fig. 3. Variation in the percentage of dissolved material and residual components in cork after organosolv treatments carried out at 160 °C for 4 h, with variable ethanol/water ratios, 0.1 M sodium hydroxide and a liquor ratio of 40:1.
(∗) Residual cork (■) Residual suberin (▲) Residual lignin (●) Residual polysaccharides.

investigation only with alkaline solutions. The following results describe the study of the optimization of the extraction process with NaOH as catalyst.

Effect of the ethanol/water ratio

The dependence of the extraction yield with the solvent composition is presented in Table 1 and Figure 3. The yield of whole extracted material increased slightly with the proportion of ethanol in the mixture, in the range 0–40 % ethanol, and then increased markedly, attaining a maximum at ca. 50 % ethanol. For higher ethanol proportions, the extraction yield decreased slightly again. However, as far as the extraction yield of suberin is concerned, a different pattern was observed. The efficiency for suberin removal increased with the proportion of ethanol, particularly in the range 40–100 %, whereas the efficiency for lignin and polysaccharides decreased in this range. Thus, although the total extraction yield reached its maximum with 50 % ethanol, the selectivity for suberin extraction was highest with 100 % ethanol. A tentative explanation could be related to the higher suberin affinity for ethanol compared with that for water. In ethanol-rich solutions, the penetration of the liquor into the cell wall and the subsequent hydrolysis/transesterification and dissolution of suberin, should occur more readily than with water-rich counterparts. With 100 % ethanol, transesterification of the ester bonds would of course predominate over hydrolysis (it should be noted that the commercial ethanol used, certainly contained traces of water). The observed decrease in the extraction yield of lignin and polysaccharides with ethanol proportions higher than 50 % was associated with the decrease of the molar fraction of water in the solution, which in turn reduced the extent of alkaline hydrolysis reactions involved in the depolymerization of lignin and polysaccharides.

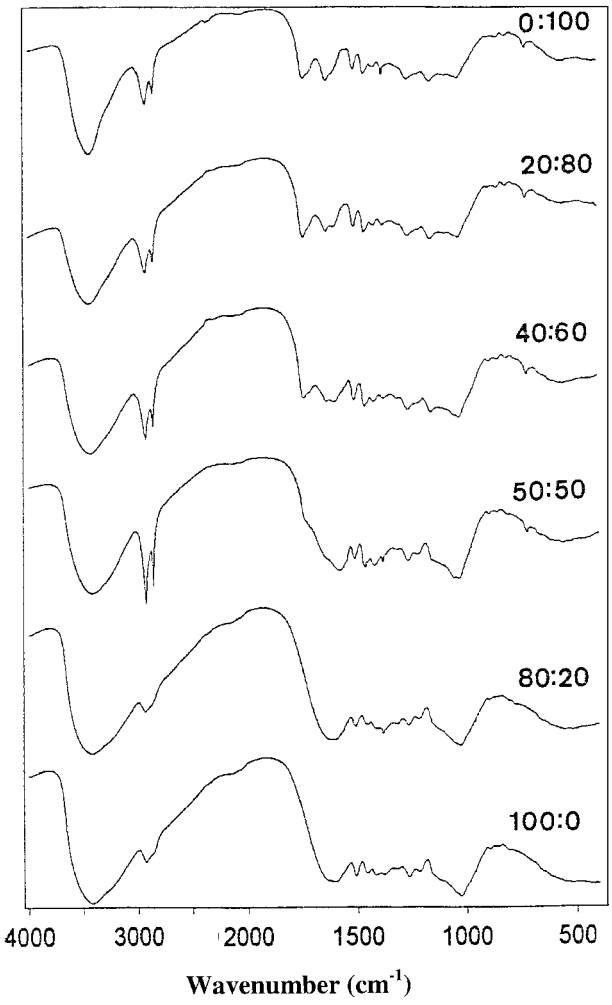

Fig. 4. FTIR spectra of residual cork from organosolv treatments carried out for 4 h at 160 °C, with different ethanol/water ratios, 0.1 M sodium hydroxide and a liquor ratio of 40:1.

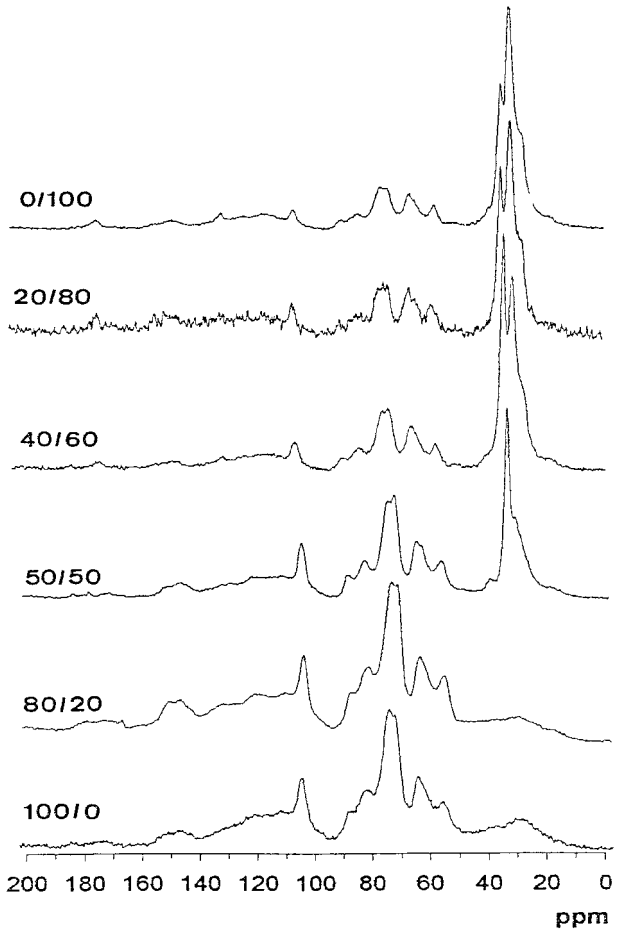

Fig. 5 ^{13}C NMR spectra of residual cork obtained with the conditions given in Figure 4.

Both effects were clearly confirmed by FTIR and NMR spectroscopy. Figure 4 shows the progressive decrease in the CH_2 and C = O vibrations, coming mainly from suberin, as the proportion of ethanol in the medium increased. The NMR spectra (Fig. 5) confirmed this trend by the overall effect of ethanol on the extraction of suberin, as shown by the progressive decrease in the resonances around 30 and 173 ppm and revealed moreover a different pattern in the decrease of the peaks at 33 and 30 ppm. Indeed, the intensity ratio I_{33}/I_{30} as a function of the medium composition showed a sharp increase between 40 and 50 % ethanol content. This specific behaviour confirmed previous observations discussed above that in the cork cell wall, suberin displays two morphologies, respectively, near the polysaccharide and lignin matrix (peak at 33 ppm attributed to less mobile methylene groups) and away from it (peak at 30 ppm for freer segments), because the latter was more easily removed than the former, which required much higher ethanol proportions. In other words, the higher the cohesive interactions binding suberin to other cork components, the harder was its detachment by the solvent, with the consequent necessity of increasing its efficiency by increasing the ethanol proportion. The effect of the composition of the ethanol/mixture on the reactivity of these two types of suberin needs further investigation.

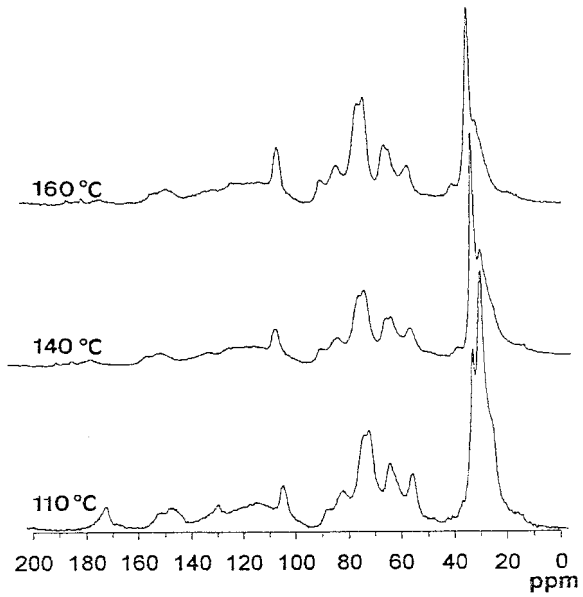

Fig. 6. ^{13}C NMR spectra of residual cork from organosolv treatments carried out for 4 h at 160 °C, at different temperatures, with 0.1 M sodium hydroxide, an ethanol/water ratio of 50/50 (v/v) and a liquor ratio of 40:1.

Effect of temperature

Table 1 gives data indicating the progressive increase in extraction yield with the optimised medium, as the temperature was raised from 110 to 160 °C. The FTIR and NMR spectra of the residues provided clear evidence that as the temperature increased, the proportion of tightly bound suberin extracted increased. Figure 6 shows how the different peaks attributed to the two types of suberin varied as a function of temperature. Again, the decrease in the intensity of both the 30 ppm and 173 ppm peaks occurred predominantly between 110 and 140 °C, whereas the peak at 33 ppm remained at a high intensity and began to decrease only at 160 °C. Again, the stronger association of the suberin sitting next to lignin and polysaccharides manifested itself by a higher resistance to extraction.

Effect of NaOH concentration

The increase in concentration of sodium hydroxide produced an increase in the extraction yield from 42.6 % to 84.4 %. The results in Table 1 show that this increase was substantial below 0.1 M and became much less pronounced above this concentration. This double regime was confirmed by the changes occurring in the FTIR and NMR spectra. Going from neutral to 0.05 M NaOH solutions produced a drastic reduction in the carbonyl vibration which disappeared already at 0.1 M concentration. As for the NMR spectra, the virtual disappearance of the peaks at 30 and 173 ppm was again recorded between 0.05 and 0.1 M NaOH, whereas the peak at 33 ppm remained strong even at the highest NaOH concentration tested (Fig. 7). These results suggest again that the suberin polymers associated to the carbohydrates-lignin matrix or to the cell walls are so tightly bound, that they resist attack even by strong ethanol/water alkaline media.

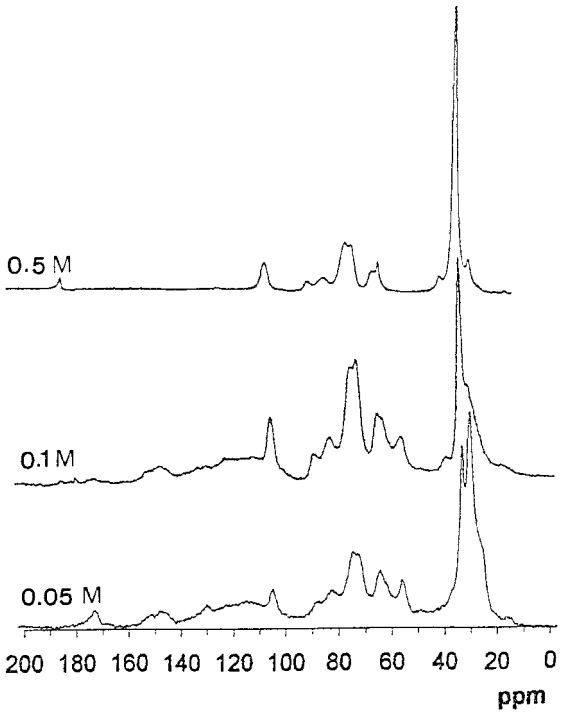

Fig. 7. ^{13}C NMR spectra of residual cork from organosolv treatments carried out for 4 h at 160 °C, with different sodium hydroxide concentrations, an ethanol/water ratio of 50/50 (v/v) and a liquor ratio of 40:1.

Effect of processing time

Increasing the extraction time from one to four hours produced an increase in yield from 35.4 % to 76.3 % (Table 1). However, this change was not linear, but rather S-shaped, indicating the occurrence of an induction period, most probably associated with the diffusion of the liquid medium inside the cork structure. The FTIR and NMR spectra of the corresponding residues indicated once again that the more loosely bound suberin, placed further away from the matrix, was extracted more rapidly than its bound counterpart.

Optimal extraction conditions

On the basis of the above systematic study of the role of the main variables related to this organosolv process, the following optimised conditions are proposed concerning the best way to extract suberin (the major and most original component of this species): extraction medium ethanol; catalyst NaOH at a 0.1 M concentration; temperature 160 °C; extraction time 4 h. More severe conditions are not interesting because the extraction of suberin *via* the transesterification/hydrolysis of its ester linkages is accompanied by the degradation of its aliphatic chains (Cordeiro *et al*. 2000).

Conclusions

The results obtained in this study suggest that organosolv fractionation of cork, which was optimised through the variation of all major parameters, may be a promising alternative separation process for its components and therefore presents a major topic of interest in terms of their subsequent valorisation. The use of different catalysts led to the conclusion that basic conditions promote both high yields of extraction and the preservation of undegraded suberin. Another interesting observation was that the linkages between suberin and the carbohydrate/lignin matrix are particularly strong and can only be released under the most severe conditions. The thorough characterisation of the extracted components constitutes the logical pursuit of this investigation and is currently in progress.

Acknowledgements

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