

CORK SUBERIN AS A NEW SOURCE OF CHEMICALS: 2. CRYSTALLINITY, THERMAL AND RHEOLOGICAL PROPERTIES

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Abstract

Suberin samples, obtained by alkaline methanolysis from cork (*Quercus suber* L.), were submitted to various physical characterizations; DSC, TGA, optical microscopy, density and rheological properties. A substantial proportion of these oligomers possessed a microcrystalline character with a melting range between 0 and 50°C. The amorphous part was liquid at room temperature and did not display a detectable glass transition upon cooling because of its wide molecular weight distribution. The viscous behaviour of suberin at room temperature was both plastic and thixotropic because of the structuring role of the microcrystals. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

The outer bark of *Quercus suber* L., commonly known as cork, is composed of suberin, its main component, contributing to about 40% of its dry weight, lignin (ca. 20%), polysaccharides (ca. 20%) and extractibles (ca. 15%) (Pereira, 1988). This peculiar chemical composition, characterized by a large contribution from a hydrophobic component (suberin, see below), together with its particular cellular structure (Pereira *et al.*, 1987), give cork excellent barrier properties for polar liquids, heat and sound (Fortes, 1990). Cork finds its main applications in stoppers, boards for insulation and decorative uses and composites (Fortes, 1990). To our knowledge, cork has not been exploited industrially up to now as a source of chemicals.

High quality cork for industrial processing is removed only from the trees which are about 40–50 years old (Fortes, 1990). Thereafter, harvesting takes place periodically in cycles of 9–10 years. The cork industry consumes annually ca. 280000 tons of raw material, of which 20–30% is rejected, mainly as cork-dust, i.e. a low-granulometry fraction without industrial interest. These tens of thousand of tons of wastes are usually burnt in the mills for energy recovery. It seems however rather uneconomical to destroy in such a manner a product which we believe worthy of a better exploitation. In fact, the unique properties of cork as material could be more rationally used in added-value applications.

For this reason, we recently started a research programme aimed at gaining a deeper insight into the structural features and the chemical properties of cork and its components (Pascoal Neto *et al.*, 1995; Cordeiro *et al.*, 1995; Pascoal Neto *et al.*, 1996; Cordeiro *et al.*, 1997a,b; Gil *et al.*, 1997; Lopes *et al.*, 1997). The end purpose of this broad investigation is to arrive at a sound knowledge of the structure–properties relationships of this natural composite which would make it possible to enhance its usage as material and to valorize it as a source of chemicals.

Within this general context, the study of suberin, which is undoubtedly the most original component of cork compared with those of other more common lignocellulosic materials, took priority in our view. The present series of articles deals therefore with its isolation, thorough characterization and possible utilization both as a specific additive and as macromonomer in polymeric compositions (Cordeiro *et al.*, 1997c).

The structure of suberin is not yet fully established. Kolattukuddy (1977) studied potato periderms and concluded that suberin, in that context, was an aliphatic polyester, similar to cutin, containing phenolic moieties like lignins, as shown in Scheme 1.

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In situ, suberin is a macromolecular network which is therefore insoluble in all solvents. It can, however, be decrosslinked by chemical processes based on the cleavage of the ester moieties. Thus, hydrolysis with aqueous alkali was used by the first cork chemists to isolate suberin from that species, but this approach was later replaced by alkaline alcoholysis (Arno *et al.*, 1981; Holloway, 1972; Holloway and Deas, 1973; Holloway, 1983; Marques and Pereira, 1987). The aliphatic depolymerization products of cork suberin obtained by these procedures were identified by GC and GC-MS techniques and found to consist mainly of 22-hydroxydocosanoic acid, 9,10-dihydroxyoctadecanedioic acid, 18-hydroxyoctadecanoic acid, 9,10,18-trihydroxyoctadecanoic acid, 9-octadecenedioic acid, 9,10-epoxyoctadecanedioic acid, 18-hydroxy-9-octadecenoic acid and 18-hydroxy-9,10-epoxyoctadecanoic acid (Holloway, 1972; Arno *et al.*, 1981).

In the previous paper (Cordeiro *et al.*, 1997a) we described a more comprehensive study of these structural features and confirmed that indeed the oligomeric portion of the hydrolysed suberin was found to bear C₁₆-C₂₄ aliphatic sequences with terminal carboxylic groups and variable amounts of OH moieties along the chains. However, we also found a distinct feature, not reported previously, consisting of the presence of a fraction possessing a much higher molecular weight. In another recent study (Cordeiro *et al.*, 1997b), we also reported the surface properties of this suberin which are important in the context of its possible applications as an additive in ink, varnishes and other coating compositions.

The present paper is a further contribution to the knowledge of suberin and deals with the characteriz-

ation of a number of bulk physical properties, including thermal, morphological and rheological features.

METHODS

Isolation of suberin

Suberin was isolated from a powder of high-quality reproduction cork (*Quercus suber* L.) with a 0.1 M methanolic NaOH solution following the procedure described previously (Cordeiro *et al.*, 1997a).

Thermal measurements

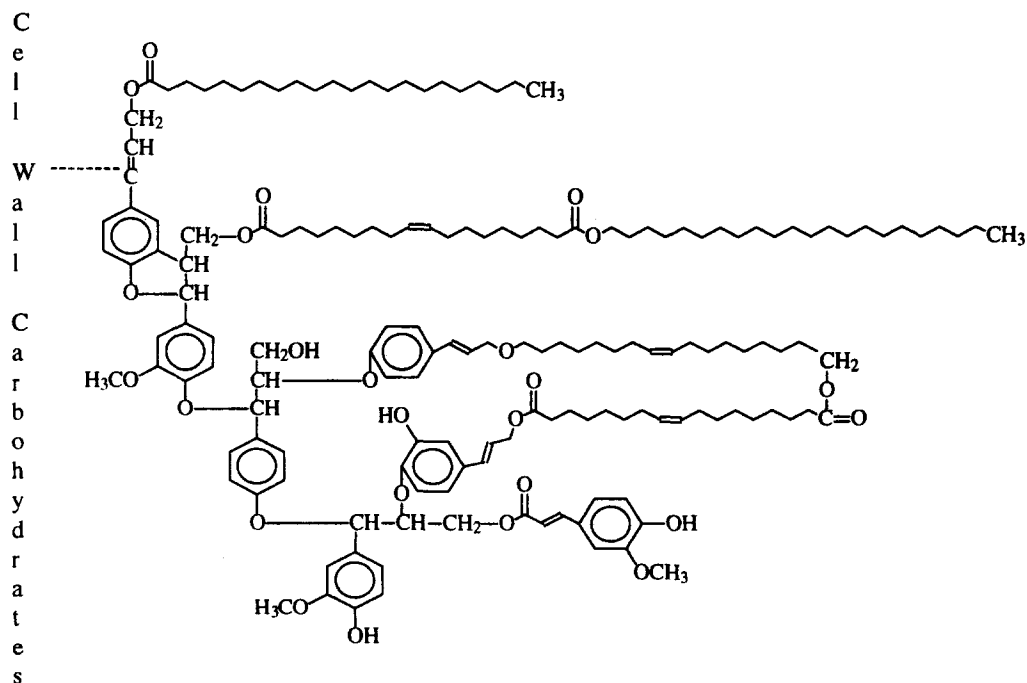
Differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA) were carried out with a Setaram DSC 92 analyzer using sealed capsules placed in a stream of dry nitrogen. Samples for DSC weighed ca. 10 mg, whereas those for TGA weighed ca. twice this. The molten DSC samples were quenched rapidly in liquid nitrogen and then analyzed by heating-cooling cycles between -150 and +150°C, at a rate of 10° (heating) and 15°C per minute (cooling).

Optical microscopy

The microscopy work was conducted with an OLYMPUS DH2 microscope equipped with a LINKHAM cooling-heating stage which allowed a temperature control of ±0.3°C. For the crystallization kinetics, a video camera was placed on the ocular system of the microscope in order to record the changes in birefringence intensity as a function of time.

Viscosity

The viscosity measurements were made with a cone-plate CARRIMED-500 rheometer working in the



Scheme 1

controlled-stress mode. The cone used had a 2 cm diameter and a 4° angle. Measurements were carried out in the temperatures range of $0\text{--}55^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$. The optimized value of the maximum imposed stress (which differed with the working temperature) was selected after testing several options. The time delays associated with the cycles involving stress increase, constant-stress periods and stress decrease were all of 1 min.

Viscoelasticity

The viscoelastic properties of suberin were measured at ambient temperature with a METRAVIB viscoanalyzer working in a compression mode.

Density

The density of suberin as a function of temperature was determined with a pycnometer calibrated with water in the $0\text{--}70^{\circ}\text{C}$ range. Three independent determinations were carried out at each temperature. The values obtained were reliable to $\pm 0.001\text{ g cm}^{-3}$.

RESULTS AND DISCUSSION

Thermal and morphological features

To our knowledge, no DSC study has previously been conducted on suberin. As shown in Fig. 1, the samples, scanned between -20 and 60°C , displayed a fairly wide endothermic peak with a minimum centred at about 40°C . The corresponding cooling thermogram, also shown in Fig. 1, exhibited an exo-

thermic peak with a maximum at 31°C . This behaviour can be readily rationalized by a melting–recrystallization cycle. Quenching the melted suberin in liquid nitrogen did not hinder crystallization as shown by the fact that the thermogram of the frozen sample was entirely similar to that shown in Fig. 1. This experiment was repeated several times and gave the same results, independent of the heating rate. It seems clear that even with very abrupt cooling, the suberin molecules are sufficiently mobile to organize themselves into (micro)crystalline domains.

In order to corroborate this important point, we called upon the observation of suberin by polarized-light microscopy with heating and cooling procedures. Fig. 2 shows a typical birefringence picture at room temperature of a suberin sample, as obtained with our isolation procedure. The presence

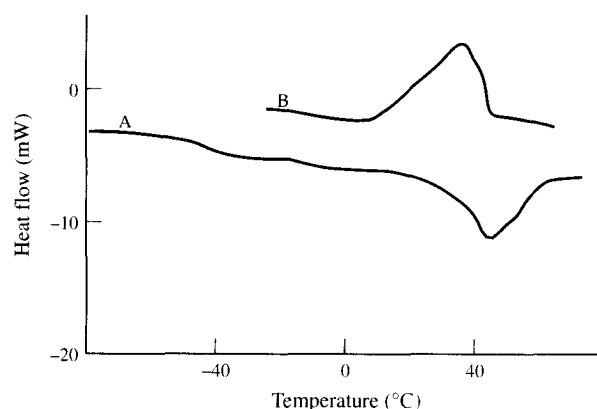


Fig. 1. DSC thermograms of suberin. A. heating; B. cooling.

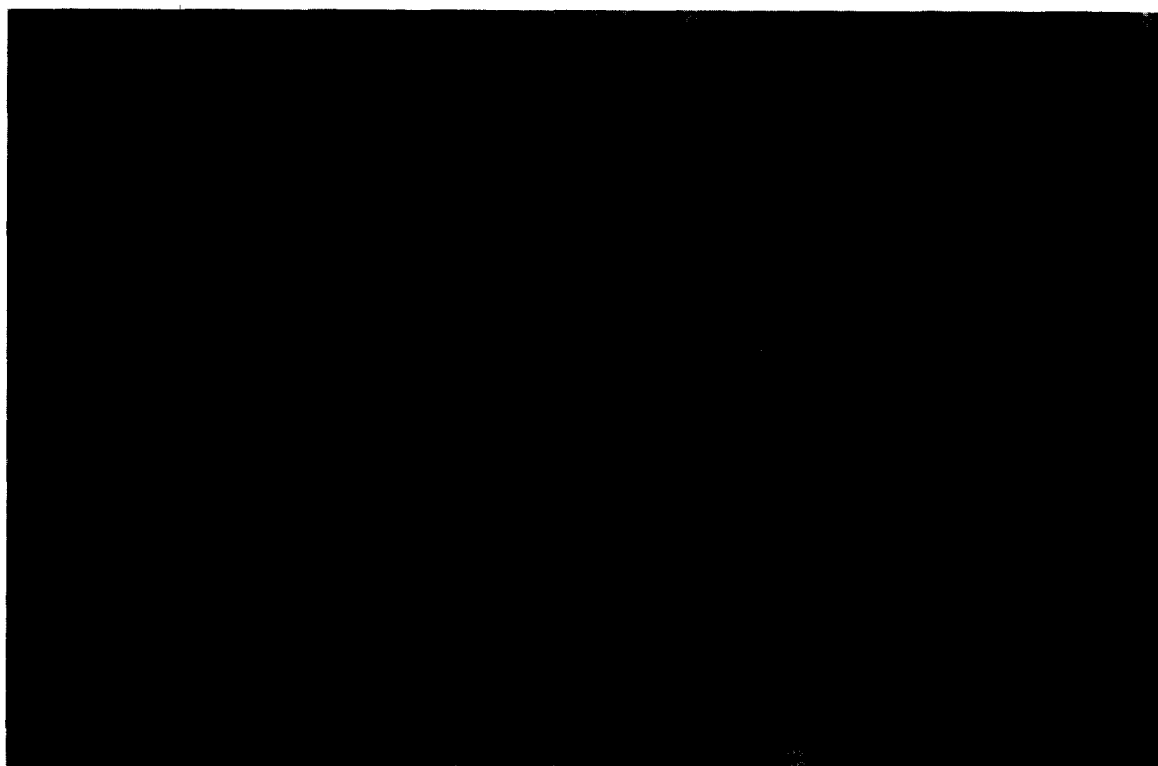


Fig. 2. A typical microscope picture ($\times 30$) of birefringence from partly crystalline suberin at room temperature.

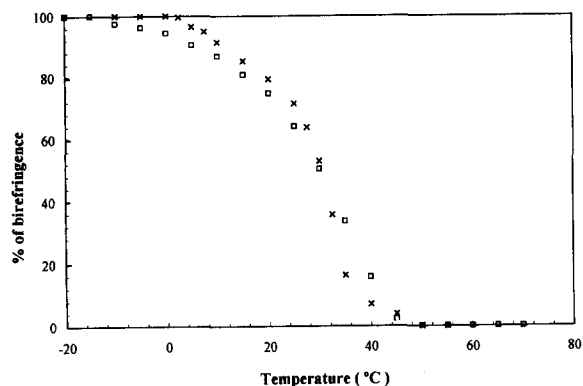


Fig. 3. Melting (X) and recrystallisation (□) of suberin as observed by the change in birefringence intensity as a function of temperature. **Note:** the 100% birefringence refers to the *maximum extent* of crystallization and *not* to the actual percentage of crystalline phase.

of an important contribution of microcrystalline phases is clearly visible. Heating these samples produced a progressive disappearance of the birefringence and at ca. 50°C the field became completely black, confirming the interpretation of the melting behaviour proposed for the DSC endotherms.

A more quantitative study of these melting features was carried out by cooling a pristine sample of suberin to -20°C and following the loss of birefringence as a function of temperature using a heating rate of $5^{\circ}\text{C min}^{-1}$. After reaching complete melting, the sample was cooled down at the same rate. Fig. 3 shows the results of this experiment which confirm the high mobility of the molecules involved in these phase changes by the remarkable reversibility of the phenomenology. The temperature range covering this melting–recrystallization cycle spanned over some 50°C , just as the correspondingly wide endothermic peak in the DSC thermograms, although the actual domains were slightly shifted. These observations confirm that our suberin samples are made up of a fairly wide distribution of molecular species, as already encountered in our previous study of their structural features (Cordeiro *et al.*, 1997a). This distribution most probably concerns variations in both molecular weight and detailed chemical composition.

The crystalline character of suberinic structures has never been reported previously, although a mention without comment of the ‘melting range’ of different suberin fractions was given in a table by Arno *et al.* (1981). The ease with which the molecules we isolated assemble to give ordered structures recalls the behaviour of paraffins, but here the presence of OH side groups (Cordeiro *et al.*, 1997a) constitute an element favouring further intermolecular organization through hydrogen bonding.

It is important to emphasize that the 100% value in Fig. 3 does not refer to total crystallization of the sample, but is simply a reference point. In fact, as clearly visible in all our microscopic observations

(see Fig. 2 as a typical example), the doubly polarized field was not entirely filled with luminous domains, suggesting the presence of amorphous (black) zones. This could not be ascribed to liquid crystallizable fractions because cooling to -150°C did not change the relative proportion of bright and dark areas compared with the situation observed at 0°C in which, typically, the black areas amounted to as much as 70% of the field.

Surprisingly however, despite numerous attempts, no clear-cut glass transition corresponding to this solid amorphous phase could be detected by DSC. It seems likely that the absence of typical Tg signals on the thermograms was not an indication of the absence of the actual glass–liquid transition, but rather a sign of a large temperature interval within which it occurred, in fact too large to give a detectable drop in the baseline. This phenomenon is common among amorphous oligomeric species possessing a wide molecular weight distribution for which the glass transition can span several tens of degrees, as with kraft lignins (Guo *et al.*, 1992).

A typical TGA thermogram of our suberin is given in Fig. 4 and shows the onset of decomposition around 300°C with more than 80% volatilization at 470°C , the residue being carbonaceous materials.

Rheological properties

Figure 5 shows a typical rheogram at room temperature which indicates that suberin follows a

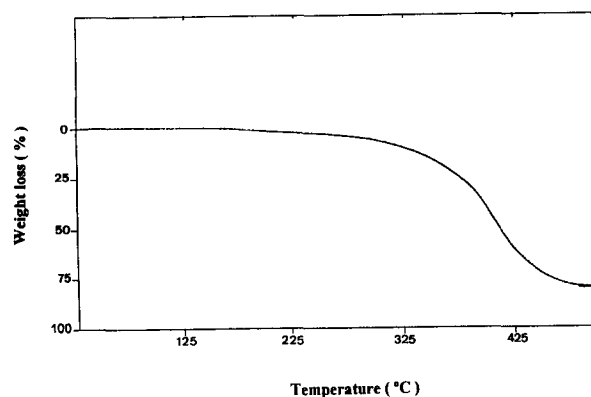


Fig. 4. TGA of suberin in a nitrogen atmosphere.

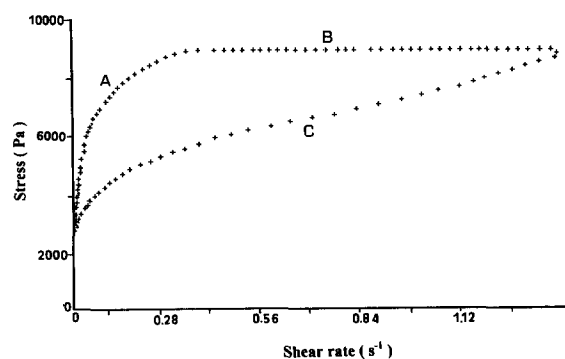


Fig. 5. A typical rheogram of suberin at 20°C . A: Increasing stress; B: Constant stress; C: Decreasing stress.

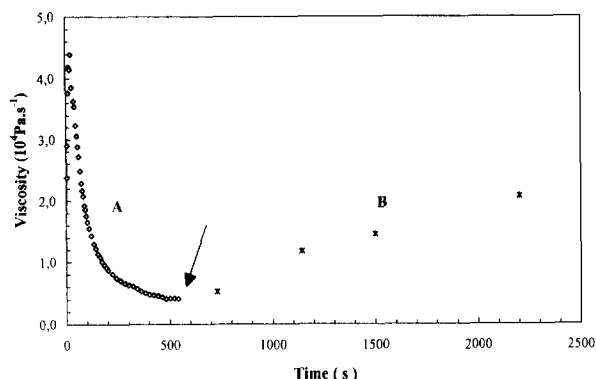


Fig. 6. Variation of suberin viscosity with time: (A) constant stress; (B) at rest. The arrow indicates when the stress was released.

Herschel–Bulkley behaviour (Richardson, 1990). The application of that viscosity model to our rheograms resulted in an excellent agreement characterized by correlation coefficients better than 0.99. This model is typically employed to fit plastic rheological responses, i.e. the existence of a yield stress, σ_s associated with intermolecular cohesive interactions, which can only be destroyed by applying a minimal shear.

Moreover, Fig. 5 also shows a strong contribution of hysteresis in the return curve. The existence of thixotropy in suberin was confirmed by the time-dependent experiment shown in Fig. 6 in which the decrease in viscosity as a function of time at constant shear stress was accompanied by a second piece of evidence given by the progressive increase in viscosity after the sample had been left at rest. Usually this type of behaviour is associated with intermolecular or interphase (solid–liquid) shear-induced destructuring followed by time-dependent restructuring at rest. The relevant question here is whether the plastic and thixotropic properties of suberin are to be attributed to the fact that at 20°C it is a mixture of liquid and crystalline phases which can establish structuring interactions. Figure 7 shows the rheograms of suberin as a function of temperature which strongly suggest that, indeed, the progressive disappearance of the crystalline particles is accompanied by a tendency towards a Newtonian behaviour. In fact at 50°C the samples displayed a

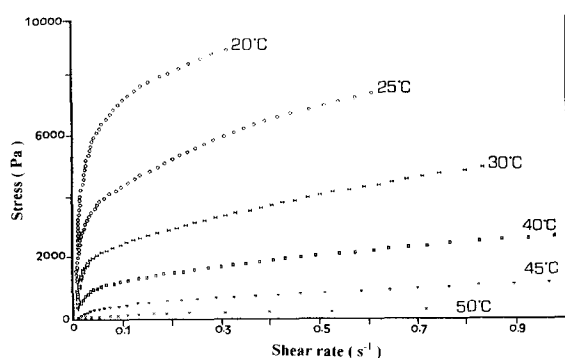


Fig. 7. Rheograms of suberin at different temperatures (increasing stress mode).

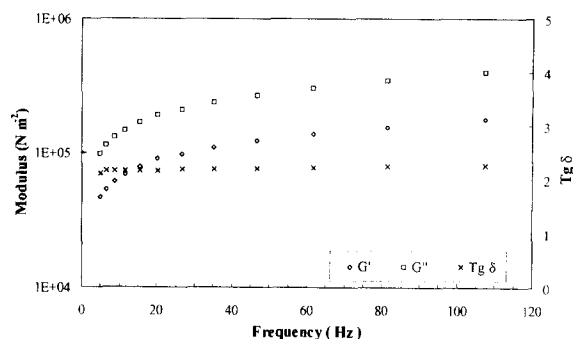


Fig. 8. Dynamic-mechanical behaviour of suberin at room temperature.

linear stress-shear rate correlation with a vanishing small σ_s . Knowing that at that temperature suberin was practically 100% liquid (Figs 1 and 3), this rheological behaviour corroborates the interpretation based on crystal–liquid structuring, but the concomitant contribution of intermolecular hydrogen bonding to this feature cannot be ruled out.

The viscoelastic properties of suberin were measured at ambient temperature and are depicted in Fig. 8. The values of the viscous, or loss, (G') and elastic, or storage, (G'') moduli only showed a modest change with frequency in the range of 5–100 Hz, except at the low values where both decreased. Their ratio ($\tan \delta$) remained remarkably constant around 2 in favour of the viscous contribution. This overall behaviour is typical of 'viscous liquids'.

Density

The change in suberin density as a function of temperature is given in Fig. 9. A number of relevant considerations can be drawn from the trends in this plot. First, since below 10°C suberin displays its highest (constant) crystallinity and above 60°C it is completely liquid (see above), the slope of the plot in Fig. 9, taken between 60 and 70°C, reflects the thermal expansion coefficient of liquid suberin and that taken between 0 and 10°C gives the same coefficient, but for the mixture containing the maximum amount of crystals dispersed into the non-crystallizable liquid. As one would expect, the latter is lower than the former.

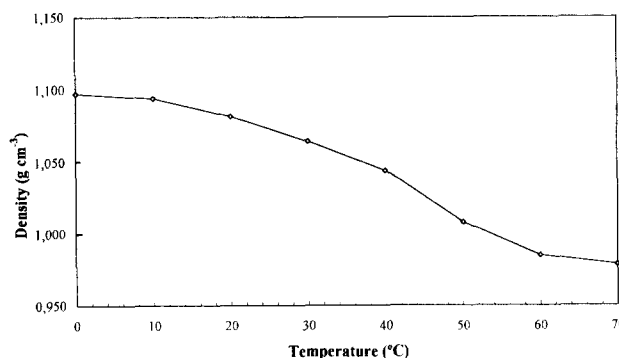


Fig. 9. Temperature dependence of the density of suberin.

Another interesting point was the more drastic drop in density between 10 and 60°C which is an excellent confirmation of the progressive melting of the crystalline phase already detected by DSC and optical microscopy.

As to the actual values, they are remarkably high, even for the liquid. In fact, they only drop below unity around 55°C, a feature that points to the existence of rather strong intermolecular cohesive interactions. Indeed, alkanes with the same molecular size have much lower densities (ca. 0.7 g cm⁻³) because they lack polar moieties and when these are introduced, e.g. two or three lateral OH groups, the corresponding macropolyols reach densities around 0.9 g cm⁻³. This increase is obviously attributed to the establishment of intermolecular hydrogen bonding, as with the case of ethylene glycol and glycerol which exhibit densities at 20°C of 1.11 and 1.26 g cm⁻³, respectively.

The values obtained here for suberin indicate very clearly that this type of interaction plays an important role and confirm the evidence obtained from other results in both this study and the previous one (Cordeiro *et al.*, 1997a).

CONCLUSION

The most relevant aspect of this investigation was the discovery of the existence of a microcrystalline component in suberin isolated from cork by alkaline methanolysis. A deeper investigation of this interesting feature is in progress particularly in order to establish whether there is any correlation between this crystalline component and one of the two distinct molecular weight fractions found in our structural study (Cordeiro *et al.*, 1997a). Another facet of this work is the strong plastic-thixotropic property of suberin around room temperature, caused by the interactions between the molecules in the liquid phase and the microcrystals suspended in it. Both these features constitute a stimulating incentive to search for applications in the realm of property-enhancing additives, e.g. in the optical quality of coating surfaces or as rheology modifiers.

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