



Composition of sugarcane waxes in rum factory wastes

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Abstract

Wastes produced during fermentation and distillation of crude sugarcane juice in rum factories were evaluated as a new source of waxes. The chemical composition of the crude wax extracted from adsorbent of the wastes on fuller's earth was studied by GC–mass spectrometry. Series of linear alkanes (C19–C33), and wax esters constitute the main components. In addition, phytosterols, tri-terpene methyl ethers, ethyl and methyl esters of fatty acids, and free fatty acids were found as minor components. Acid (pre-dominance of C16 and C18) and alcohol portions (C26–C32) of the wax esters were analysed after saponification.

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1. Introduction

Sugarcane wax has always been a matter of interest, due to its industrial applications, in particular in the cosmetic and pharmaceutical industry (Taylor, 2000). It is a potential substitute for costly carnauba wax widely used in cosmetics, foods and pharmaceuticals. In addition, sugarcane wax is also a source of long chain primary aliphatic alcohols, which find applications as cholesterol-lowering products (Laguna Granja et al., 1999; Mas et al., 1999). During the agro-industrial process, a large part of the wax is dissolved in the crude juice, then removed in the wastes during the subsequent defecation-clarification step (filter cake from sugar refinery) or distillation (fermentation wastes and vinasses). Wax recovery from filter cake is well documented (Paturau, 1989) as are the relevant methods and techniques at the laboratory or industrial level (Lamberton and Redcliffe, 1965; Parfait, 1997; Askew et al., 1999). In contrast, to the best of our knowledge, fermentation wastes and vinasses from distilleries and rum factories have never been exploited despite their potential importance as a low cost source of wax considering the enormous resources of

raw material. Indeed, about 20 l of vinasses are produced for each litre of rum. Vinasses are generally disposed of in landfills, causing nuisance and pollution due to their high organic and mineral content (Navarro et al., 2000). In order to reduce the polluting charge, a process has been developed by a local distillery in Guadeloupe, which consists of organic matter adsorption by filtration of the vinasses through fuller's earth. In this context, it appears interesting to study the chemical content of these adsorbents in order to evaluate the potential of vinasses as a new source of sugarcane wax. Among the constitutive molecules, several types of compounds from different origins were expected: molecules unmodified by alcoholic fermentation, molecules modified by biochemical transformations or by biologic agents acting during fermentation, and molecules originating from the latter agents themselves. The objectives of this study were to isolate and chemically characterize the classes of compounds of the raw wax obtained by extraction of the adsorbents.

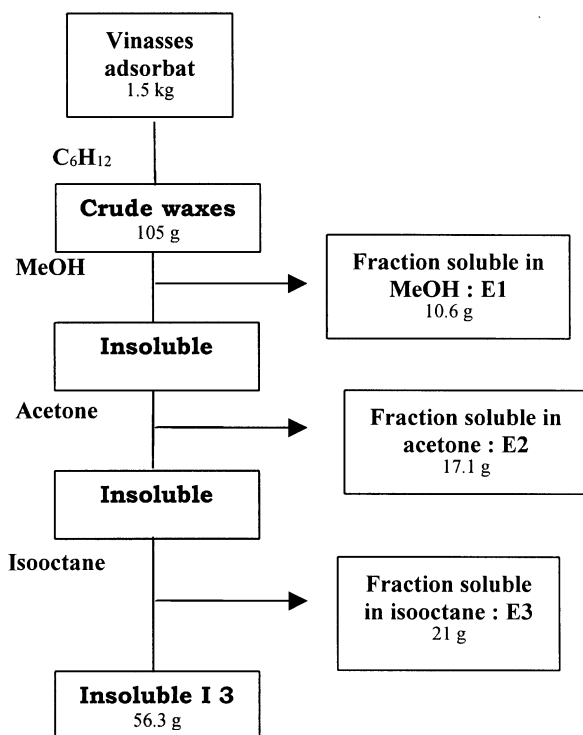
2. Results and discussion

The crude waxy material obtained by extraction of the adsorbent with cyclohexane (see Experimental) was triturated sequentially with solvents of decreasing polarity, i.e. MeOH, acetone and isooctane (Scheme 1).

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The yields and the characteristics of the four resulting fractions E1–E3 and I3 are summarized in Table 1. Each fraction was analyzed by TLC (SiO_2) to monitor the classes of compounds, then chromatographed on silica gel columns followed by preparative TLC. The main results of the chemical analyses are summarized in Table 1.



Scheme 1. Extraction and fractionation of the crude wax.

2.1. MeOH-extracted fraction (E1)

This fraction accounted for 10% of the crude wax. It was made up of fatty acid esters, pentacyclic triterpenoids, sterols and free fatty acids, which were chromatographed on silica gel (Table 2). Triterpenes and methyl and ethyl fatty acid esters were eluted first as a mixture (9:91, 7% yield). The presence of palmitic acid isopentyl ester (1.5%) was also detected. Gas chromatography showed the palmitic esters to be the major products (73%). The content and composition of the fatty esters are closely linked not only to the yeast, but also to the conditions of fermentation and distillation processing. The distillation apparatus can create slow reactions of esterification due to the heating, the residence time in the apparatus, and the catalytic function of the copper parts (Fahrasmane and Ganou-Parfait, 1997). The predominance of ethyl esters can be related to the ethanol widely produced during fermentation. Formation of isopentanol during the fermentation step had been reported by Fahrasmane and Ganou-Parfait (1997) and could explain the presence of the isopentyl ester. Pentacyclic triterpenoids accounted for 9% of the fraction and less than 1% of the methanolic extract. Crugallin (3%), arundoin (trace amounts), cylindrin (4%), skimmione (2%) were identified by GC/MS and comparison with literature data for *Saccharum officinarum* leaf wax (Smith and Martin-Smith, 1978a) and Cuban sugarcane wax (Bryce et al., 1967). From these results, it seems that fermentation and distillation do not alter the structure of triterpenes, except for skimmione, which had not been isolated from filtercake and

Table 1
Composition of the 4 fractions obtained from the crude wax (for details, see Tables 2–3)

Fractions	Yields (%)	Aspect	Saponification index	CC (SiO_2) solvent systems	Type of compounds	R_f values (SiO_2) TLC	Relative portions (%)	Unidentified (%)
E1	10	Pasty, orange coloured	174	C_6H_{12} to AcOEt	Methyl and ethyl esters Triterpenoids Sterols Free fatty acids	(C_6H_{12} /AcOEt: 8:2) 0.87 (C_6H_{12} /AcOEt: 8:2) 0.33 (C_6H_{12} /AcOEt: 8:2) 0.6 (C_6H_{12} /AcOEt: 6:4)	7 ^a 2 37	54
E2	16	Paraffin, dark-green	67	C_6H_{12} /AcOEt 95:5 to 50:50	Alkanes Sterols	0.85 (C_6H_{12} / CH_2Cl_2 : 95:5) 0.33 (C_6H_{12} /AcOEt: 8:2)	7 9	84
E3 (saponified)	20	Grey powder	95	C_6H_{12} / CH_2Cl_2 30:70 to 0:100	Alkanes α,β -Unsaturated aldehydes Alcohols Fatty acids	0.85 (C_6H_{12} / CH_2Cl_2 : 95:5) 0.44 (C_6H_{12} / CH_2Cl_2 : 8:2) 0.32 (C_6H_{12} / CH_2Cl_2 : 5:5) 0.6 (C_6H_{12} /AcOEt: 6:4)	8 31 36	25
I3 (saponified)	54	Grey powder	80	C_6H_{12} / CH_2Cl_2 90:10 to CH_2Cl_2 /AcOEt: 70:30	Alkanes α,β -Unsaturated aldehydes Alcohols Fatty acids	0.85 (C_6H_{12} / CH_2Cl_2 : 95:5) 0.44 (C_6H_{12} / CH_2Cl_2 : 8:2) 0.32 (C_6H_{12} / CH_2Cl_2 : 5:5) 0.6 (C_6H_{12} /AcOEt: 6:4)	7 32 28	33

^a For methyl, ethyl esters and triterpenoids together.

Table 2
Fatty acids, esters and triterpenes, sterols composition (%) of E1 and E2

Carbon number	Fatty acids (%) E1	Methyl esters (%) E1	Ethyl esters (%) E1	Isopentyl esters (%) E1	Triterpenes (%)	Sterols (%)	
						E1	E2
12:0	–	–	3	–			
14:0	Tr	–	Tr	–	Crusgallin: 3 Skimmione: 2	β -Sitosterol : 53 Stigmasterol : 31	β -Sitosterol : 47 Stigmasterol : 23
15:0	1	–	–	–			
16:0	57	8	65	1.5	Arundoin: Tr	Campesterol : 16	Campesterol: 22
18:0	13	2	1	–			β -Sitostanol: 2
18:1	15	4	Tr	–	Cylindrin: 4		Campestanol: 1
18:2	2	–	–	–			Cholesterol: Tr
20:0	2	–	–	–			Delta-22-stigmasterol: Tr
22:0	1	–	–	–			
24:0	Tr	–	–	–			
Unidentified	9			8 ^a			

^a For methyl, ethyl, isopentyl esters and triterpenoids together.

could result from the acid hydrolysis and oxidation of taraxerol methyl ether.

Sterols were also isolated from E1, accounting for 2%. β -Sitosterol (53%), stigmasterol (31%) and campesterol (16%) were identified by GC/MS after trimethylsilylation (Table 2). Δ^5 -Avenasterol and Δ^5 -stigmastadiene-3-ol, previously reported in local filter press cake (Parfait, 1997), and 4 α -monomethyl- Δ^7 -sterols isolated from leaf waxes of Cuban sugarcane (Osske and Schreiber, 1965) were not detected in our extract.

Stigmasterol and β -sitosterol have been previously obtained from sugarcane by a three-step process involving saponification with NaOH, acidification by HCl and several extractions (Mitra and Kapadia, 1988). They were shown to have a significant stimulative effect on ethanol production when added to alcoholic fermentation media (Bourgeois and Fahrasmane, 1988). Phytosterols constitute useful raw materials for the pharmaceutical and cosmetic industries (Zayas et al., 1997).

Free fatty acids were also isolated from the methanolic extract (Table 2). They constituted the most abundant fraction (37% of E1), the composition of which was determined by GC/MS after methylation. Saturated acids accounted for 74% of the total free acids, with palmitic acid (C16:0) as the predominant constituent (57%), followed by stearic acid (C18:0, 13%). In addition, two unsaturated acids were found in this fraction, namely oleic (C18:1, 15%) and linoleic acid (C18:2, 2%) (Table 2).

2.2. Acetone-extracted fraction (E2)

This fraction, which represents 16% of the crude wax, was analysed on TLC, then chromatographed on silica gel. Alkanes were eluted first. They accounted for 7% of

the E2 fraction and 1.1% of the total crude wax (Table 1). Their composition was determined by GC, and comparison with standards. Odd and even carbon-numbered *n*-alkanes in the C20–C29 range were found, with a large predominance of heptacosane (31%). In addition, minor amounts of branched alkanes were detected (Table 3).

Phytosterols were also isolated accounting for 9% of E2 (1.4% of the crude wax). They were characterized by GC/MS as trimethylsilyl ether derivatives. β -sitosterol, stigmasterol and campesterol were the main sterols, with respectively 47, 23 and 22% (Table 2). Small amounts of β -sitostanol (2%), campestanol (1.5%), Δ^{22} -stigmasterol and cholesterol (trace amounts) were also detected. The sterol distribution of this fraction is similar to those previously described for sugar filter cake (Parfait, 1997).

2.3. Isooctane-extracted and insoluble fractions (E3, I3)

In an attempt to recover additional alkanes and fatty esters, the acetone insoluble material was triturated in isooctane, leading to partial dissolution. The soluble (E3, mp 68–70 °C, 20% yield) and insoluble (I3, mp 80 °C, 54% yield) parts were separated and analysed by NMR and mass spectrometry. Inspection of the spectra showed that both were mainly constituted of wax esters and alkanes. In order to identify fatty acid and alcohol moieties, saponification in ethylene glycol was performed. Alkane, alcohol and fatty acid compositions were then determined by GC/MS after separation on silica gel column (Table 3). In addition, α,β -unsaturated aldehydes were detected by ¹H and ¹³C NMR before and after saponification (data not shown), but could not be identified by GC/MS. Presence of long chain aldehydes have been previously mentioned in

Table 3
Alkanes, alcohols and fatty acids composition (%)

Carbon number	E2	Alkanes (%)		Alcohols (%)		Fatty acids (%)	
		E3	I3	E3	I3	E3	I3
10	–	–	–	–	–	Tr	Tr
12	–	–	–	–	–	1	1
14	–	–	–	–	–	1	1
15	–	–	–	–	–	1	1
16	–	–	–	–	–	55	42
17	–	–	–	–	–	1	1
18	–	–	–	–	–	10	7
18:1	–	–	–	–	–	–	9
18:2	–	–	–	–	–	–	7
19	–	–	–	–	–	1	–
20	2	–	–	–	–	2	1
21	2	–	–	–	–	Tr	–
22	2	–	–	–	–	1	1
23	6	–	–	–	–	Tr	1
24	7	–	–	–	Tr	2	3
25	15	–	2	–	–	Tr	1
26	21	–	2	6	5	1	1
27	31	17	27	Tr	Tr	1	1
28	3	1	2	81	63	4	7
29	4	18	16	Tr	–	Tr	–
30	–	1	1	7	5	2	2
31	–	16	14	Tr	–	Tr	–
32	–	2	3	4	Tr	1	–
33	–	24	18	Tr	–	Tr	–
34	–	–	7	Tr	–	1	–
35	–	12	–	–	–	–	–
Unidentified	7	9	8	2	27	15	13

sugarcane wax (Lamberton, 1965). Although the same classes of compounds were identified in the soluble (E3) and insoluble (I3) fractions, differences appeared for the distribution of the individual alkanes and acids (Table 3).

The hydrocarbon fractions were eluted first (8% for E3; 7% for I3). Odd carbon-numbered *n*-alkanes ranging from C25 to C35 were found here. The profiles were dominated by tritriacontane for E3 and heptacosane for I3. These results are in agreement with the alkane profile previously reported for leaf wax of *Saccharum officinarum*, which was shown to be in the same range, but dominated by C29 and C31 (Smith and Martin-Smith, 1978b).

Fatty primary alcohols resulting from the saponification were trimethylsilylated prior to gas chromatographic analysis. A large predominance of octacosanol (C28) was observed in both E3 and I3, along with small amounts of C26, C30 and C32. The potential application of the mixture of long chain aliphatic primary alcohols isolated from sugarcane wax, also called polycosanol, has been well documented in the literature, as cholesterol-lowering products (Pons et al., 1997; Laguna Granja et al., 1999; Mas et al., 1999; Menandez et al., 1994). Moreover, polycosanol can be used for the

treatment of atherosclerotic complications (Laguna Granja et al., 1999).

Medium and long chain acids were also isolated from the saponification products of E3 and I3 with, as expected, even carbon-numbered acids predominating (Table 3). The individual acids ranged from C10 to C34, with a large predominance of palmitic in both E3 and I3. Stearic (C18:0) and octacosanoic acid (C28:0) were also present in both (4–10%), while oleic (C18:1, 9%) and linoleic (C18:2, 7%) acids were only found in I3.

In conclusion, adsorbates of vinasses produced during fermentation and distillation of the crude sugarcane juice in rum factories can be used as a new source of sugarcane waxes. Series of linear alkanes (7%), and wax esters constitute the main components of such adsorbates. Phytosterols (2%), triterpene methyl ethers, ethyl and methyl fatty esters (1%), and free fatty acids (4%) were also present. This composition is in good agreement with the results of the literature for filtercake wax. However, some differences appear in relative abundances, which could be due to the distillation and fermentation steps (Parfait et al., 1972), the variety and origin of the sugarcane plant (Smith and Martin-Smith, 1978a) as well as the technology used to obtain the wax (Garcia et al., 1998).

3. Experimental

3.1. Plant and material

The fermentation and distillation wastes came from the treatment of the 1997 sugarcane harvest in Guadeloupe by a local distillery. They were filtered on fuller's earth, leading to adsorbats. The adsorbats were dried at 50 °C in a drying oven, then ground before use leading to a whitish powder.

3.2. General

Mps are uncorr. GC was performed on a Delsi DN 200 under the following conditions: fused silica capillary column, CP Sil 5 CB 25 m×0.25 mm id, film thickness 0.25 µm, temp. prog: 150–320 °C at 6 °C min⁻¹, injector temp. 240 °C (splitless mode), FID temp. 300 °C, carrier gas He (0.9 bar). For GC/MS, 70 eV EIMS were recorded under the same conditions using a micromass Auto-spec-Q set to scan the mass range 20–850 amu at 0.37 s per decade. Samples were introduced to the MS via a Hewlett Packard HP 5890 series II (direct inlet) working in the split mode (splitting ratio 1:50).

3.3. Derivatization

Trimethylsilyl (TMSi) derivatives of sterols and alcohols were prepared using HMDS/TMSiCl (NF ISO 6799). Fatty acids were methylated using MeOH/BF₃ (NF EN ISO5509). Fatty esters were converted into their methyl esters using the same process after saponification (see Section 3.5).

3.4. Extraction of waxes

The crude wax mixture was extracted from the ground adsorbat (1.5 kg) in a soxhlet apparatus, using cyclohexane at room temperature until obtaining a deep coloration of the solvent (during about 20 h). The extract was concentrated under low pressure leading to brown paraffin (105 g, 7%: wt.% relative to the total mass of adsorbat, acid value 13 and saponification value 91). The resulting crude wax was extracted again in a soxhlet type apparatus by decreasing polarity solvents: MeOH, acetone and isooctane (100 ml, room temperature for the two first ones and by stirring at 80 °C and decantation for the last one). This led to three extracts (E1–E3) and insoluble material (I3), as summarized in Scheme 1, which were examined by analytical TLC (SiO₂, constituents detected by spraying with concentrated H₂SO₄ and charring, or DNPH). Fractions E1–E2 were submitted to preparative column chromatography on silica gel (Merck 0.040–0.063 mm), and finally purified on preparative plates (SiO₂, Merck 1 mm). A CH₂Cl₂/MeOH: 80:20 mixture saturated with

NH₄OH was used to recover the compounds from the adsorbent after development. Fractions E3 and I3 were saponified, then chromatographed on silica gel.

3.5. Saponification of E3 and I3

Extracts (30 g) and KOH (5.6 g) in ethylene glycol (200 ml) were stirred for 2 h at 150 °C. The unsaponifiable matter was removed by extraction with cyclohexane. Then HCl was added (to pH 1), leading to an emulsion which was extracted in a soxhlet apparatus with cyclohexane. Fatty acids and fatty alcohols were recovered after evaporation of the solvent.

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