Chemistry of Natural Compounds, Vol. 44, No. 5, 2008

EVALUATION OF VINASSES FROM SUGARCANE MOLASSES DISTILLATION AS A NEW SOURCE OF SUGARCANE WAX

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UDC 547.91+665.3

Molasses, a by-product of sugar manufacturing, are the most common raw material for rum manufacturing. During the fermentation and distillation process, vinasses are produced in large quantities and disposed in landfills. In this study, they were evaluated as a new source of sugarcane wax. The chemical composition of the wax was studied by GC-Mass spectroscopy. A series of n*-alkanes (C_{23}–C_{33}) and ethyl and methyl esters of fatty acids (palmitate and oleate are the predominant), of phytosterols (stigmasterol,* β*-sitosterol, campesterol), free fatty acids ($C_{12:0}$–$C_{36:0}$), and triglycerides constitute the main components. In addition, 2-ketones (C_{27}–C_{33}), aldehydes (C_{28}, C_{32}, C_{34}), ketosteroids (derivatives of stigmasterol,* β*-sitosterol, and campesterol), and fatty alcohol acetates (alcohol moiety: C_{28}, C_{30}, C_{32}) were found as minor products.*

Key words: molasses, vinasses (wastewaters), distillery, sugarcane wax, chemical composition.

Crude rum is produced by fermenting and distilling sugarcane juice or, most often, molasses, which are the residues of sugarcane processing. During this process, a wastewater called vinasses (or spent wash, distillery slops) is produced in huge quantities. Depending on the cane quality and the industrial process, approximately 20 L of vinasses are released per liter of pure alcohol [1]. Vinasses from molasses are the most difficult products to dispose because of their low pH, dark brown color, high ash content, and high percentage of dissolved organic and inorganic matter [2]. In addition to its nauseating odor, this residue, when carelessly released, significantly pollutes the natural environment, particularly rivers [3].

Currently, methanation [4] and physical treatments (incineration, sewage farming) are possible methods of reuse.

Sugarcane wax has always been a matter of interest due to its industrial applications, in particular in the cosmetic and pharmaceutical industries [5]. In the present work, we described the extraction and the chemical composition of sugarcane wax obtained from a new raw material: vinasses from sugarcane molasses.

The waxy material obtained with chloroform was chromatographed on silica gel, leading to 5 main fractions (I-V). Each of them was analyzed by NMR in order to determine the main class components, R_f values, and chemical compositions (determined by GC/MS see Table 1). Yields are calculated from the mass of the dried solid extract.

The NMR spectrum of fraction I showed the typical pattern for *n*-alkanes [4.5%; R_f 0.65 (heptane/CH_2Cl_2:8/2)]. Its composition was determined by GC and compared with authentic standards (Table 1). Odd and even *n*-alkanes ranging from C_{23} to C_{33} were found, with C_{27} being the major component (25.3%). The significant amounts of even alkanes C_{24} (10.1%), C_{26} (13.1%) and C_{28} (6.4 %) can be pointed out. These values are in agreement with literature for sugarcane cuticle waxes [6].

Free *n*-fatty acids [(fraction V; 8.2% in crude wax, R_f 0.32 (CH_2Cl_2/AcOEt:7/3)], fatty acid esters [fraction II; R_f 0.85 (heptane/CH_2Cl_2:5/5) FAE – 4%, ketosteroids – 2%], and triglycerides [fraction III; 2.4%, R_f 0.8 (CH_2Cl_2)] were isolated from the waxy material and identified by NMR. Their compositions were determined by GC after transmethylation (triglycerides) or GC/MS (free fatty acids and esters) (Table 2). Methyl, ethyl, and isopentyl esters contribute 10%, 84%, and 6% respectively, to the total esters.

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 0009-3130/08/4405-0552

TABLE 1. Alkanes, Fatty Alcohol Acetates, Ketones, and Aldehydes Compositions

Carbon	Alkanes	Fatty alcohol acetates	Ketones	Aldehydes	Carbon	Alkanes	Fatty alcohol acetates	Ketones	Aldehydes
	%					%			
15	-	-	Tr.	-	29	11.0	-	27.3	-
23	6.4	-	-	-	30	3.2	11.6	-	-
24	10.1	-	-	-	31	6.0	-	32.6	-
25	12.1	-	-	-	32	1.8	5.2	-	17.8
26	13.1	-	-	-	33	4.1	-	31.1	-
27	25.3	-	8.9	-	34	-	-	-	17.8
28	6.4	83.2	-	64.0					

Tr.: trace.

TABLE 2. Fatty Acid Esters, Triglycerides, and Free Fatty Acids (FFA) Composition, %

Acids	Esters			Triglycerides	FFA	Acids	FFA	Acids	FFA
	methyl	ethyl	isopentyl						
10:0	-	Tr.	-	-	-	18:2	6.0	28:0	5.6
12:0	-	5.4	12.3	-	3.2	20:0	1.5	29:0	1.2
14:0	-	2.3	8.2	-	1.7	21:0	Tr.	30:0	3.3
15:0	-	-	-	-	Tr.	22:0	1.4	31:0	Tr.
16:0	25.0	67.4	59.4	55.0	40.9	23:0	Tr.	32:0	2.0
16:1	-	-	-	-	4.5	24:0	1.6	33:0	Tr.
17:0	-	Tr.	-	-	Tr.	25:0	Tr.	34:0	1.7
18:0	75.0	23.0	19.3	17.0	15.9	26:0	1.9	36:0	1.1
18:1	-	Tr.	-	22.0	4.4				

Tr.: trace.

For the three classes, Table 2 shows that the predominant fatty acid chain lengths were C_{16} and C_{18} (saturated and unsaturated chains). The free fatty acid composition is very similar to the fatty acid ester distribution. This may indicates that chemical or enzymatic transformations occurring during alcoholic fermentation would have probably converted free fatty acids into their homologs by esterification with methanol, ethanol, or isopentanol produced in fermentation media [1]. Clinical studies have demonstrated that very-high-molecular-weight aliphatic primary acids purified from sugarcane wax lower cholesterol and prevent plasma lipoprotein peroxidation [7]. Mixtures of di- and triglycerides with chain lengths from C_{30} to C_{60} have been previously reported for Cuban sugarcane filter muds [8].

After isolation by preparative TLC, sterols [(fraction IV 2.4%, R_f 0.8 (CH_2Cl_2)] were identified by GC/MS as β-sitosterol (38.4%), stigmasterol (33.4%), and campesterol (22.3%) along with traces of cholesterol, an animal sterol, sometimes found in small amounts in plants [9]. Ergosta-8(14)-dien-3β-ol (2.9%), a hydrogenated derivative of ergosterol, which is the main sterol in yeast, was previously mentioned by Rezanka [10] as a sterol of *Saccharomyces cerevisiae*. This sterol composition can be compared to literature data [11]. Phytosterols have been shown to be an alternative method in lowering plasma cholesterol levels [12].

Stigmasta-3,5-dien-7-one (45.8%), stigmasta-3,5,22-trien-7-one (28.9%), campesta-3,5-dien-7-one (25.2%), and traces of cholesta-3,5-dien-7-one were identified in fraction II by GC/MS and literature data [13]. It is worth noting that this ketosteroid composition is similar to the sterol distribution obtained from fraction IV. Recently, Georges et al. [14] found the same ketosteroids from press mud sugarcane wax.

Some minor components were detected by GC/MS (Table 1). Small amounts of even-numbered fatty alcohol acetates (alcohol moieties: C_{28}–C_{32}, C_{28} widely represented), of even-numbered aldehydes (C_{28}, C_{32} and C_{34}, C_{28} being the major aldehyde), and of odd 2-ketones (C_{27}-C_{33}, C_{31} and C_{33} being the most important) were identified. Fatty alcohol acetates probably result from reactions between acetic acid (produced during alcoholic fermentation) and long-chain free fatty alcohols. These form a part of sugarcane wax from press mud [15]. Concerning the aldehydes, it has been previously reported that octacosanal (in polymeric form) was the major aldehyde in sugarcane wax [16]. 2-Ketones could come from the enzymatic transformation of fatty acids and/or triglycerides [17]. Previously, only α,β-unsaturated ketones had been mentioned in the literature by Stransky et al. [18] as components of sugarcane filter muds.

From this study, we can conclude that the vinasses from molasses distillation can be used as a new source of sugarcane wax. Analysis of the extracted waxy material showed some similarities to sugarcane waxes from the usual origins (cuticle, filter cake). Our results showed that 1 L of alcohol produced in rum processing, thus 20 L of vinasses, could lead to the recovery of approximately 3.4 g of crude wax. The huge volume of vinasses released every year and the necessity of finding uses for this highly polluting waste can make their reuse for producing sugarcane wax very attractive.

EXPERIMENTAL

Analytic TLC plates and silica gel for chromatographic columns (silica gel 60, 0.040–0.063 mm) were purchased from Merck. GC was performed on a Hewlett Packard 5890 series II under the following conditions: fused silica capillary column, CP sil 5 CB (25 m length, 0.25 mm diameter, 0.12 mm film thickness), temperature program 150–320°C at a rate of 6°C/min, then 320°C for 15 min, injector temperature set at 280°C (splitless mode), FID detector set at 300°C. For sterols, the temperature program was 270–320°C at a rate of 3°C/min, then 320°C for 30 min. Helium was used as the carrier gas (10 psi). 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 to 850 a.m.u at 0.37 s per decade. Samples were introduced to the MS via a Hewlett Packard HP 5890 series II (direct inlet) working in split mode (split ratio was 1:50). Identification of individual components was accomplished by comparison with authentic standards or with a collection of authentic spectra (NIST). Fatty acids were methylated with MeOH/BF_3, and sterols silylated with BSTFA + 1% TMCS. Triglycerides were converted into their methyl esters using KOH/MeOH (NF T 60.233 norm).

Extraction of the Wax. Fresh wastewaters from sugarcane molasses distillation were obtained from a rum factory in Guadeloupe (French West Indies). They were stored at –15°C until use in order to prevent microbial degradations. They were mixed with sea-sand (used as an adsorbent), then dried and crushed. 1.5 kg of the dried mixture (sand weight accounted for about half of the mixture) was extracted with $CHCl_3$ in a Soxhlet type apparatus until a deep coloration of the solvent was obtained (about 14 h). The chloroformic solution was evaporated under low pressure to give 6.8 g of a brown viscous liquid. After washing with water and drying on $MgSO_4$, the chloroform layer was evaporated under reduce pressure to give the final waxy material (dark brown solid: 1.8 g) which was chromatographed on silica gel (heptane/CH_2Cl_2:8/2 to pure AcOEt). Separation was monitored by analytic TLC (silica plates, constituents detected by UV 254 nm and/or spraying with concentrated H_2SO_4 and charring).

ACKNOWLEDGMENT

We are grateful to the CESAMO, Université Bordeaux I, for recording the GC/MS spectra.

REFERENCES

1. L. Fahrasmane and B. Ganou-Parfait, *De la Canne au Rhum*, INRA, Paris, 1997.
2. D. Pant and A. Adholeya, *Bioresource Technol.,* (2006), doi: 10.1016/j.biortechn. 2006.09.027.
3. A. R. Navarro, M. Del C Sepulveda, and M. C. Rubio, *Waste Manag.*, **20**, 581 (2000).
4. R. Seth, S. K. Goyal, and B. K. Handa, *Resour. Conserv. Recycl.*, **14**, 79 (1995).

5. A. K. Taylor, *Chem. Innov.*, **30**, 45 (2000).
6. Z. Kranz, J. Lamberton, K. Murray, and A. Redcliffe, *Aust. J. Chem.*, **13**, 498 (1960).
7. M. Noa, S. Mendoza, and R. Mas, *J. Med. Food*, **8**, 345 (2005).
8. K. Stransky, M. Streibl, and J. Zajic, *SOFW-J.*, **105**, 411 (1979).
9. S. Xu, G. Patterson, and K. Schmid, *Phytochemistry*, **25**, 1883 (1986).
10. T. Rezanka, *J. Chromatogr. A*, **598**, 219 (1992).
11. P. Goswami, H. Singh, and J. Baruah, *Curr. Sci.*, **53**, 917 (1984).
12. M. Moghadasian, *Life Sci.*, **67**, 605 (2000).
13. A. Balde, S. Apers, T. De Bruyne, H. Van den Heuvel, M. Claeys, A. Vlietinck, and L. Pieters, *Planta Med.*, **66**, 67 (2000).
14. P. Georges, M. Sylvestre, H. Ruegger, and P. Bourgeois, *Steroids*, **71**, 647 (2006).
15. B. K. Guita, G. K. Gupta, J. L. Suri, and C. K. Atal, *Res. Ind.*, **30**, 95 (1985).
16. P. Kolattukudy, *Chemistry and Biochemistry of Natural Waxes*, Elsevier Scientific Pub. Co., New York, 1976.
17. T. Yagi, M. Kawaguchi, T. Hatano, A. Hatano, T. Nakanishi, F. Fukui, and S. Fukui*, J. Ferment. Bioeng.*, **71**, 93 (1991).
18. K. Stransky, J. Zajic, M. Streibl, L. Doubravova, and K. Ubik, *SOFW-J.*, **104**, 47 (1978).