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Characterization of Products from Sugar Cane Mud

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Abstract. An efficient methodology for the extraction and purification of mixtures of high molecular weight primary aliphatic alcohols, phytosterols and fatty acids from sugar cane mud is described, as well as the determination of the qualitative and quantitative composition of these mixtures.
Keywords: *Saccharum officinarum*, sugar cane mud, sugar cane wax. aliphatic primary alcohols, phytosterols, fatty acids.

Resumen. Se describe una metodología eficiente para la extracción y purificación de mezclas de alcoholes alifáticos primarios de alto peso molecular, fitosteroles y ácidos grasos a partir de la cachaza de caña de azúcar, así como la determinación de la composición cuali y cuantitativa de dichas mezclas.
Palabras clave: *Saccharum officinarum*, cachaza de caña de azúcar, alcoholes primarios alifáticos, fitoesteroles, ácidos grasos.

Introduction

Sugar cane mud (*Saccharum officinarum*, L.) is a residue of the Sugar Cane Industry obtained by vacuum filtration of the grounds that flocculate when sugar cane juice is clarified, with the objective of to eliminate non-soluble impurities contained on it. Mud composition is very variable, but in general it has a high content of soil, cane trash, saccharides and sugar cane wax [1].

Sugar cane wax is found near the sugar cane stem nodules, in the cuticle, its main components are high molecular weight esters, polyesters, fatty acids, dicarboxylic acids, aldehydes, ketones, alcohols and hydrocarbons [2]. During the sugar cane grinding process, most of the sugar cane wax is present in the juice, the former is separated in the vacuum filtration process of the clarified juice, forming part of the mud.

Different extraction procedures of phytosterols from sugar cane mud oil [3], and higher primary aliphatic alcohols (HPAA) [4] and fatty acids [5] from refined wax, have been patented; however, an efficient methodology for the selective isolation of the above mentioned mixtures from mud has not been described before.

The biological activity and applications of mixtures of HPAA [6], phytosterols [7] and fatty acids [8] are well known.

Results and discussion

Crude wax was extracted from mud by refluxing in *n*-hexane for 1 h, the extract was filtered off and the filtrate was evaporated under reduced pressure. Three samples (100 g each) arising from the "Antonio Guiteras", "Majibacoa" and "Camilo Cienfuegos" Cuban Sugar Cane Factories were used. Extracted waxes (41.3 % from mud) were homogeneously blended in equal proportions, obtaining a green solid (dripping temperature: 76-78 °C). The ratio of esters, HPAA, phytosterols and fatty acids in the crude wax were 42.8, 15.4, 1.9 and 35.5 %, respectively [9].

In order to increase the contents of HPAA and fatty acids in the crude wax, the latter was saponified using NaOH in ethanol for 1.5 h. The amount of NaOH depends on the wax saponification index [9] (73 mg KOH / g of wax in this case), using 20-25 % excess base. The required saponification time was determined by monitoring the disappearance of the IR carbonyl stretching bands for esters and acids (1743 and 1712 cm^{-1}, respectively), and the concomitant appearance of O-H stretching band (3300 cm^{-1}) and symmetric and asymmetric stretching C-O bands of the carboxylate group (1562 and 1416 cm^{-1}, respectively).

The saponified mixture precipitates in the ethanolic phase when room temperature is reached, which was filtered off under reduce pressure. The filter contains high concentration of HPAA and fatty acids salts, while phystosterols remain solved in the filtrate. From these two fractions, products are extracted and purified.

Tables 1, 2 and 3 show the qualitative and quantitative composition of the HPAA, phytosterols and fatty acids.

The present methodology is the result of optimizing parameters to achieve an efficient extraction of the main components from sugar cane mud, such as saponification conditions (base, solvent, temperature and reaction time), as well as reflux conditions (solvent, reflux time and number of extractions). Also, it is worth mentioning that all chromatographic separations were carefully explored in order to find the best reliable peak resolutions. In conclusion, hereby is described a very useful procedure to take advantage of sugar cane mud as an important source of high molecular weight esters and alcohols, fatty acids and phytosterols.

Table 1. Qualitative and quantitative composition of the HPAA mixture.

Identified component	Condensed formula	Contents (%)[a]
n-tetracosanol	*n*-$C_{24}H_{49}OH$	1.7
n-hexacosanol	*n*-$C_{26}H_{53}OH$	5.5
n-heptacosanol	*n*-$C_{27}H_{55}OH$	2.9
n-octacosanol	*n*-$C_{28}H_{57}OH$	60.0
n-nonacosanol	*n*-$C_{29}H_{59}OH$	2.9
n-triacontanol	*n*-$C_{30}H_{61}OH$	8.6
n-dotriacontanol	*n*-$C_{32}H_{65}OH$	1.7
n-tetratriacontanol	*n*-$C_{34}H_{69}OH$	3.3
Total	—	86.6

[a] Determined by gas chromatography.

Experimental part

Melting points and dripping temperature (uncorrected) were determined with a Buchi 535 capillary melting point apparatus. IR were recorded on a Perkin-Elmer 1600 spectrophotometer. Gas chromatography was performed in a Philips PU4600 Chromatograph. To determine HPAA composition a glass capillary column fused silica (2.4 m × 4 mm) with OV-101 (3 %) and the FID-FID detection dual system was used. Nitrogen was employed as the mobile phase at 40 mL / minutes, tube temperature was 230-320 °C, carrying gas flow 38.5 mL/minutes and hydrogen flow 385 mL/minutes. Sigma standars of HPAA were used. HPAA were derivatized by using *N*-methyl-*N*- tetramethylsilil-trifluoroacetamide dissolved in pyridine. To determine fatty acids composition, a steel column (Chromosorb W-AM, 100-120 mesh, 2 m × 3 mm) with PEGA (20 %) and H_3PO_4 (1 %) was used. Nitrogen was employed as the mobile phase at 40mL / minutes. Sigma standars of fatty acids were employed. HPLC was carried out in a Philips PU 44110 (Unicam) UV-visible detector, Spherisorb S50DS1 (RP-18), methanol / water (99:1) 1 mL / minute at λ = 205 nm. Sigma standars of phytosterols were used.

Saponification of crude wax. Crude wax (100.0 g) finely chopped was mixed with 10.0 g of NaOH in 95 % EtOH (1 L). The mixture was refluxed for 1 h, filtered at high temperature and the filtrate was rinsed with 500 mL of boiling EtOH. When the filtrate reached room temperature, the precipitated wax was separated by means under reduced pressure filtration.

Extraction and purification of HPAA. Saponified wax in acetone (500 mL) was refluxed for 10 min, then 0.5 g of charcoal was added, heated to boiling during 5 min and filtered while hot. When the filtrate reached room temperature, it was again filtered under reduced pressure and recrystallized from 95 % EtOH. The product was dried in a vacuum oven at 50 °C to give the HPAA fraction as white amorphous powder (78 %), mp 82-84 °C.

Extraction and purification of phytosterols. The ethanolic solution obtained at the end of the vacuum filtration in the saponification stage was diluted in equal volume of water, allowed to stand for 1 h and then was centrifugated for 15 min at 6000 rpm. The liquid was separated and the solid was washed with H_2O / EtOH (3:1) and centrifugated again using the same conditions. The product was recrystallized from 95 % EtOH and dried in vacuum oven at 50 °C, to give the phytosterols fraction as white crystals (90 %), mp 132-134 °C.

Table 2. Qualitative and quantitative composition of the phytosterols mixture.

Identified component	Contents (%)[a]
stigmasterol	27.5
fampesterol	30.0
β-sitosterol	31.6
Total	89.1

[a] Determined by HPLC.

Table 3. Quantitative and qualitative composition of fatty acids mixture.

Identified component	$C_{n:i}$[a]	Contents (%)[b]
myristic	$C_{14:0}$	0.7
palmitic	$C_{16:0}$	20.2
stearic	$C_{18:0}$	4.3
oleic	$C_{18:1}$	4.4
linoleic	$C_{18:2}$	1.3
linolenic	$C_{18:3}$	2.1
arachidonic	$C_{20:0}$	0.2
n-tetracosanoic	$C_{24:0}$	0.7
n-hexacosanoic	$C_{26:0}$	0.3
n-octacosanoic	$C_{28:0}$	25.6
n-nonacosanoic	$C_{29:0}$	1.2
n-triacontanoic	$C_{30:0}$	15.0
n-dotriacontanoic	$C_{32:0}$	8.3
n-tetratriacontanoic	$C_{34:0}$	11.1
total		95.4

[a] *n*: number of carbon atoms in the alkyl chain. *i*: number of non-saturated bonds.
[b] Determined by Gas Chromatography.

Extraction and purification of fatty acids. The H_2O / EtOH solution obtained during phytosterols separation was refluxed for 5 min and filtrated while hot. The filtrate was adjusted to pH 3.5 using HCl (10 %), the precipitate was separated under reduced pressure filtration, recrystallized from 1,2-dichloroethane and dried in a vacuum oven at 50 °C, to give the fatty acids fraction as white amorphous powder (64 %), mp 80-82 °C.

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