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Volatile Compounds Obtained by the Hydrodistillation of Sugarcane Vinasse, a Residue from Ethanol Production

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Compostos voláteis obtidos por hidrodestilação da vinhaça de cana-de-açúcar, um resíduo da produção de etanol

Resumo: A vinhaça é o principal resíduo das indústrias de produção do etanol. Gerada em grande quantidade, ela é constituída por água, compostos orgânicos e inorgânicos e é usada extensivamente como fertilizante na agricultura, promovendo melhora na qualidade do solo e no rendimento de culturas. Entretanto, possui elevado potencial poluidor, elevada taxa de matéria orgânica, podendo levar a contaminação do solo e de águas, além do risco de lixiviação por componentes minerais. Neste estudo, os componentes voláteis da vinhaça foram obtidos por hidrodestilação em aparelho Clevenger e analisados por cromatografia a gás acoplada à espectrometria de massa (CG-EM). A mistura obtida apresentou aspecto de cera e sua análise por CG-EM indicou que é rica em ácidos graxos, álcoois e ésteres. Os componentes majoritários foram álcool feniletílico (22,28%), ácido mirístico (17,45%), ácido palmítico (15,81%), palmitato de etila (8,99%) e hexadecanol (6,06%).

Palavras-chave: Vinhaça de cana-de-açúcar; voláteis; produção de etanol; CG-EM.

Abstract

Sugarcane vinasse is the main by-product from ethanol production industries. Generated in large quantities, it is composed by water, inorganic and organic compounds and is extensively used as fertiliser in agriculture, promoting improvements in soil quality and crop yield. However, it has high pollution potential, as the high concentration of organic matter can lead to contamination of the soil and water, in addition to the risk of leaching of the mineral components. In the current study, the volatile components of vinasse were obtained by hydrodistillation using a Clevenger apparatus, then analysed by gas chromatography-mass spectrometry (GC-MS). A waxy material was obtained, and the GC-MS analysis indicated that vinasse is rich in fatty acids, alcohols and esters. The major components were phenylethyl alcohol (22.28%), myristic acid (17.45%), palmitic acid (15.81%), ethyl palmitate (8.99%) and hexadecanol (6.06%).

Keywords: Sugarcane vinasse; volatiles; ethanol production; GC-MS.

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Volatile Compounds Obtained by the Hydrodistillation of Sugarcane Vinasse, a Residue from Ethanol Production

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1. Introduction

Vinasse is the residue obtained from the ethanol distillation process, characterized by its high biochemical and chemical oxygen demand. It is used as a fertiliser for agricultural and animal pasture areas. Depending on the application time, area and concentration, its agricultural application has been shown to contribute to the soil nutrient content, reduced land degradation and improved crop yield.[1] However, the leaching of mineral components, such as potassium and nitrate, combined with the high level of organic matter in vinasse, provides a source of contamination of soil, surface water, groundwater and the surrounding environment if applied in excess to a particular area.[2,3] Specific recommendations should be followed for each region in order to prevent excessive use and subsequent leaching of minerals.[3] Furthermore, studies have shown that the dilution of vinasse in water (50% v/v) has mutagenic effects on

Tradescantia pallida.[4] Therefore, it has been recommended that use of vinasse be carried out with caution, despite being economically viable.[4] When the vinasse is not used in fertigation, some alcohol production industries have instead deposited it in regions known as “sacrifice areas”. These areas pose a great risk to groundwater and rivers by potential leaching.[5] The vinasse from sugarcane is rich in organic matter and mineral components,[1] and its composition may vary depending on several factors, including the raw material used to produce ethanol, the distillation process employed,[4] collection method, and the technology used for the production of ethanol and determination of soil quality.[6]

In addition to several studies that have evaluated the use of vinasse as a fertiliser, several other studies have evaluated its: potential use for bioremediation of soil contaminated with diesel oil,[7] mutagenic activity,[4] use in combustion processes,[8] treatments to reduce the pollution potential (e.g., biodigestion)[5,9] and the volume generated in the ethanol production process,[5] chemical composition,[10,11] use in animal feed,[11,12] biogas production,[13] biohydrogen production,[14] allelopathic activity for weed control,[15] and the evaporation and concentration of vinasse for use as fuel.[6]

As vinasse is a waste product generated in high quantities by the alcohol industry (10–18 L vinasse per litre of alcohol produced),[3] it is a threat environment. So, research has focussed on new applications of vinasse and technologies to reduce the volume produced and its pollution potential.[5]

Vinasse is a rich source of organic compounds, and these compounds could be analysed and identified by gas chromatography coupled to mass spectrometry (GC-MS). Previous studies have used GC-MS to identify several organic compounds in the residues derived from the distillation of molasses and sugarcane juice used to produce rum.[15,16]

A more detailed knowledge of the chemical composition of sugarcane vinasse generated by ethanol production is important for determining its properties, and could be useful for identifying new uses for this by-product. To our knowledge, no studies in the literature have investigated the volatiles present in sugarcane vinasse produced as a by-product of ethanol production. Therefore, the objective of this study was to obtain the volatile fraction of vinasse by hydrodistillation and to characterize its chemical composition using GC-MS.

2. Materials and methods

2.1. Material collection

Vinasse samples were collected from an alcohol distillery located in the municipality of Buritizal, São Paulo, Brazil (20°11’29” S, 47°42’18” W), on the morning of 26 August, 2013. The samples were stored under refrigeration until extraction of the volatile components.

2.2. Extraction of volatile compounds

Approximately 250 mL of vinasse was extracted by hydrodistillation for 4 hours using a Clevenger apparatus. The mixture obtained was extracted with 10 mL dichloromethane, after that the organic layer was separated. This fraction was dried with anhydrous sodium sulphate, then concentrated by evaporation at room temperature, and kept in a closed vial at -10°C for further analysis.[17] The resulting waxy mixture was dissolved in dichloromethane at a concentration of 5 mg/mL and analysed by GC-MS.

2.3. Analysis of volatile compounds

The analysis was performed using a gas

chromatograph coupled to a mass spectrometer (QP2010; Shimadzu, Kyoto, Japan) equipped with a DB-5 J&W capillary column (5% phenyl 95% polydimethylsiloxane; 30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min, and the detector and injector temperatures were set to 220 and 240°C, respectively. The injection volume was 1 µL of the solution at a concentration of 5 mg/mL, and the split ratio was 20:1. The oven temperature was programmed from 60 to 246°C at a rate of 3°C/min and then kept at this temperature for 38 min. The electron impact energy was set at 70 eV, and fragments from *m/z* 40 to 650 were collected.[18]

2.4. Identification of the volatile constituents

Identification of the volatile components of the vinasse was achieved based on the arithmetic index (AI) and the mass spectra of reference compounds from the Nist08, Wiley139, Wiley229 and ShimDemo, and Shim2205 libraries.[19,20] Some patterns were used in the analysis, they are marked with an asterisk (*) in Table 1. The AI was calculated based on alkane standards (C8–C30) using the following equation: $AI\ (x) = 100\ PzC + 100[(t\ (x) - t\ (Pz))/t\ (Pz + 1) - t\ (Pz))]$ where t is the retention time in minutes; x is the unknown compound, PzC is the carbon number of the alkane Pz that runs before x, and Pz + 1 is the alkane that runs after x.[19] Quantification was obtained after normalization of the peak areas in the total ion chromatogram. The results represent average values of three experiments. Compounds of concentration < 0.9 % were not considered. Similarity indexes < 92 % were also rejected.

3. Results and discussion

The extraction of vinasse by hydrodistillation using the Clevenger apparatus promoted volatilization and dragging of compounds in the mixture, resulting in a product with a waxy consistency, here it was called vinasse wax. As we were unable to find any other studies that have used this methodology to extract vinasse, this wax obtained from the hydrodistillation process was considered as a new material.

For each litre of vinasse, 17.5 g of dry matter was obtained, and 36.0 mg was obtained by steam distillation. Therefore, the vinasse wax had a yield of 0.2%. Figure 1 shows the chromatogram for the vinasse wax obtained by GC-MS.

Table 1 shows the volatile compounds identified in vinasse according to Figure 1. Table 2 presents the classification of compounds by their functional groups.

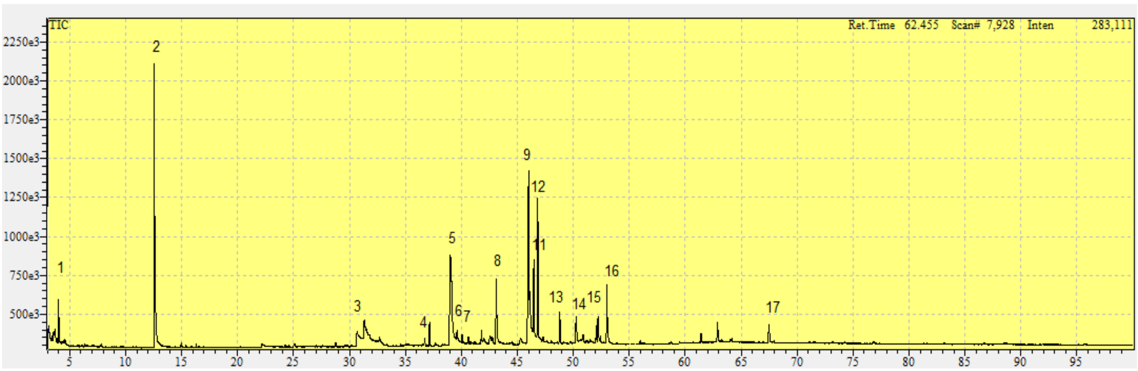

Figure 1. Chromatogram of vinasse wax

Table 1. Volatiles of vinasse wax

Peak	Compounds	AI literature	AI calculated	(%)	Identification method
1	Furfural	828	836	1.84	a, b, c, d
2	2-Phenylethanol	1120	1120	22.28	b, c, d, e
3	Dodecanoic acid (lauric acid)	1565	1566	2.68	a, c, d
4	Pentadecanal	1716	1714	1.25	a, b, c, d, e
5	Tetradecanoic acid (myristic acid)	1770	1766	17.45	b, d, e
6	Pentadecan-1-ol	1773	1782	1.78	a, b, c, d, e
7	Ethyl tetradecanoate (ethyl myristate)	1795	1794	0.9	a, b, c, d
8	Hexadecan-1-ol	1874	1881	6.06	a, c, d
9	Hexadecanoic acid (palmitic acid)*	1959	1965	15.81	a, b, c, d, e
10	Ethyl *E*-hexadec-9-enoate (ethyl palmitoleate)	1975	1972	4.30	b, c, d, e
11	N.I		----	4.57	a, b, c
12	Ethyl hexadecanoate (ethyl palmitate)	1992	1991	8.99	a, c, d
13	2-phenylethyl dodecanoate (2-phenylethyl laurate)	2053	2055	2.00	d, e, f
14	N.I	---	----	1.85	a, b, c
15	Ethyl (*Z*)-octadec-9-enoate (ethyl oleate)	2171	2166	2.06	b, c, d, e
16	Ethyl octadecanoate (ethyl stearate)	2196	2192	4.57	a, c, d
17	Heptacosane*	2700	2700	1.64	a, c, d
				Total identified (%) 93.61	

AI, arithmetic index; N.I., not identified. Identification method: (a) AI compared with the AI described by Adams;[19] (b) AI compared with the NIST standard reference data;[20] (c) mass spectrum compared with the mass spectral database by Adams;[19] (d) mass spectrum compared with the Shim2205 and Wiley libraries spectra; (e) mass spectrum compared with the NIST standard reference data;[20] (f) AI compared with the Pherobase database.[21]

Table 2. Classification of compounds present in the vinasse wax according to their functional groups

Functional groups	Vinasse wax (%)
Alcohols	30.12
Aldehydes	3.09
Esters	22.82
Long chain alkanes	1.64
Fatty acids	35.94
Not identified	6.42

The major components identified in the volatile phase of vinasse were phenylethyl alcohol (22.28%), myristic acid (17.45%), palmitic acid (15.81%), ethyl palmitate (8.99%) and hexadecan-1-ol (6.06%), as shown in Table 1. Figure 2 shows the formula of the major compounds identified.

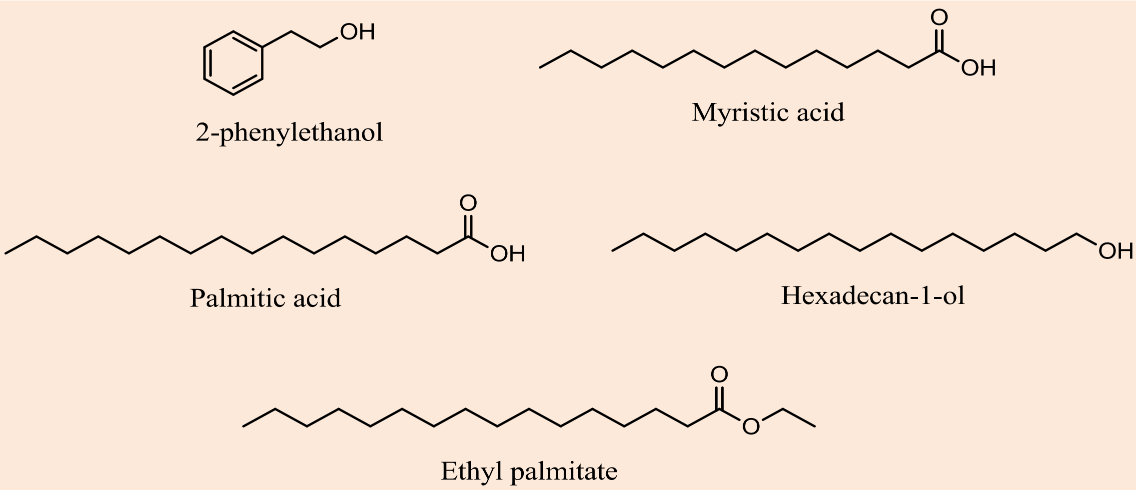

Figure 2. Main volatile compounds of vinasse wax

Evaluation of the compounds present in the waxy material, separated by their functional classes, showed that fatty acids were present in the highest percentage (35.94%), followed by alcohols (30.12%) and esters (22.82%). Long-chain alkanes and aldehydes were the least abundant components. The free fatty acids found in the vinasse wax contained saturated carbon chains, with myristic acid (17.45%) being the predominant fatty acid, followed by palmitic acid (15.81%) and lauric acid (2.68%). Ethyl palmitate (8.99%), ethyl stearate (4.57%) and ethyl palmitoleate (4.30%) were the predominant esters. The high concentration of esters in the wax may be explained by the conversion of fatty acids to esters via esterification reactions that occur during fermentation.[22] All esters identified in the wax were ethyl esters. This may be related to the high availability of ethanol generated during fermentation.[15] The constituents of the vinasse wax may have originated from sugarcane, or from products formed as a result of reactions that occur at different stages of the manufacturing process.

Two studies that have characterized the resulting waste from rum production were found in the literature.[15,16] In one of these studies, extractions were carried out using organic solvents on the dry residue from the distillation of molasse. Subsequent GC-MS analysis of these extracts revealed that long-chain alkanes (C_{23}–C_{33}), methyl and ethyl esters (predominantly palmitate and oleate), phytosterols (stigmasterol, β-sitosterol and campesterol), free fatty acids $C_{12:0}$–$C_{36:0}$ (saturated and unsaturated chains, mainly C16 and C18) and triglycerides were the main components. Long-chain aldehydes, ket-2-ones, ketosteroids and acetates of fatty alcohols were the minor components.[16] Other study analysed the residue from the distillation of sugarcane juice derived from rum manufacturing. The same classes of constituents were present when the dry residue was extracted with organic solvents and the extract analysed by GC-MS. However, there were variations in the proportion of each of the classes of compounds and the components identifed.[15] Discrepancies observed between literature studies and the present work may be explained by differences in the industrial process, extraction method and solvents used. However, some classes of compounds, such as long-chain alkanes, esters, free fatty acids, aldehydes and alcohols, have been found in all residues, regardless of ways of obtainment.

The Clevenger steam distillation method, which only extracts the volatile components of vinasse, innovatively employed in this work, allowed us to obtain a new material from vinasse derived from ethanol production. This highlights the simplicity of the methodology used.

The fatty acids observed in the vinasse wax are also present in high concentrations in the essential oils of different plant species, obtained by the same extraction method. These essential oils have demonstrated antimicrobial and antifungal activities.[17,23] The biological activities of volatile natural products obtained by steam distillation are mainly related to the predominant constituents, or from synergistic action of the major and minor compounds present in the mixture.[24] Several fatty acids have been studied alone or in mixtures, and have shown antimicrobial and antifungal activities against different microorganisms.[25,26,27] Furthermore, these fatty acids have high commercial value.

Alcohols represented 30.12% of the composition of the volatile phase of vinasse, and 22.28% corresponded to phenylethyl alcohol. Pentadecanol and hexadecanol represented 7.84% of the composition. Long-chain alcohols have proven antimicrobial activity.[28] Phenylethyl alcohol is a compound with the odour of roses, found naturally in the essential oils of some vegetables and foods such as tea, coffee, cocoa, beer and cheese.[29] It is an important aromatic alcohol, widely used in cosmetics, perfumes and food. Phenylethyl alcohol can be produced by chemical synthesis, however, its high relevance to a number of industries, combined with its high commercial price,[29] has led to the evaluation of alternative methods of biotechnological production.[30]

Regarding the esters identified in the vinasse wax, ethyl palmitate was the predominant compound in this class, making up 8.99% of the total composition. This compound has anti-inflammatory,[31] acaricide[32] and antimicrobial activities.[33]

The aldehydes presented in the vinasse wax included only pentadecanal (1.25%) and furfural (1.84%). Pentadecanal is an aliphatic aldehyde found in the essential oils of some plants.[34,35] Furfural is used in the chemical industry as an intermediate for the production of various materials such as nylon, lubricants, solvents, adhesives, pharmaceuticals and plastics.[36] This compound can be produced from the degradation of sugars during ethanol production. When exposed to high temperatures and an acidic environment, polysaccharides (hexoses and pentoses) can decompose to compounds including hydroxymethylfurfural and furfural, respectively.[37]

A future direction for this research is the evaluation of the chemical composition of vinasse wax produced by other alcohol industries. The chemical composition of vinasse from sugarcane varies from one industry to another,[4,6] therefore, it is possible that the composition of the volatile wax also differs. Further studies are then necessary to have a general profile for vinasse waxes.

4. Conclusions

Alcohols, aldehydes, esters, fatty acids and long chain alkanes were classes of compounds in the vinasse wax. The major components identified in the volatile phase of vinasse were 2-Phenylethanol, myristic acid, palmitic acid, ethyl palmitate and hexadecan-1-ol. Thus, the wax obtained from sugarcane vinasse is a source of industrially important compounds with recognized biological activities, justifying the assessment of this material in different biological assays, including its toxicity.

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