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Chemical Characterization of *N*-Hexane Extract Obtained from Lignocellulose Residual Contained in Agroindustrial Wastes

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Abstract

The chemical composition of *n*-hexane extractives from lignocellulose residual contained in different agroindustrial wastes was studied. The *n*-hexane extract, which accounted for 1.6% of total lignocellulose residual weight, was analysed by ^{1}H-NMR and gas chromatography/mass spectrometry. The most predominant compounds identified were aliphatic hydrocarbons (lineal alkanes, branched alkanes, alkenes). Additionally, terpenes, ketones, fatty alcohols, fatty acids and steroids were also found together in minor amounts. The lipophilic compounds in *n*-hexane extracts in three lignocellulosic wastes, which is highly valuable information for a more complete industrial utilization of these lignocellulosic materials.

Keywords

Wheat Straw, Maguey Bagasse, Sugarcane Bagasse, *N*-Hexane Extract

1. Introduction

The development of agroindustry has led to the generation of great quantities of waste, which affects the environment. The production of some foods such as cereals, breath and sugar is increasing worldwide, therefore the generation of waste by this as well. Wheat straw is an abundant residue from wheat production in many countries. The average amount of wheat straw is 1.3 - 1.4 kg/kg of wheat grain [1], with a global production of 755.8 million tons wheat grain worldwide in 2016/2017 according to FAO 2018. The production of sugar from sugarcane generates two fundamental residues: bagasse and straw; these residues are produced in great quantities, generating approximately 280 million tons per year

[2]. Another waste generated is the maguey bagasse, which reaches accounts until 40% of the processed agave, generating approximately 105,000 tons per year [3] in Mexico.

There is a growing necessity to transform the current agricultural industry focused on the production of only food in an extensive industry that also satisfies other sectors such as biofuels or added-value chemicals [1]. In this context, lignocellulose biorefineries appears, in which the waste generated by the agroindustry can be used [4]. These residues can be fractionated or processed to obtain compounds with high added-value [5].

One of those is the extraction of compounds with low polarity solvents (*n*-hexane, ethyl ether, ethyl acetate) that could be used by other industries [6] as in perfumery, cosmetics and medicinal. The extracts obtained are rich in different groups of compounds [7]; such as hydrocarbons, acids and fatty alcohols [8] [9], esters and phytosterols among other compounds [1] [10] [11] [12] [13] [14].

Several studies have demonstrated the wide range of application of *n*-hexane extracts from different samples. Firstly *n*-hexane is economical (centimes per liter) and secondly its functionality: is difficult to find a substance that competes with it to do the same function at a similar cost. That is, we find a very cheap solvent, which works very efficiently, is easily removed, can be recycled, and does not accumulate in the body or the environment. This makes it really difficult to substitute for less dangerous alternatives.

The extract obtained from seeds of *Jatropha curcas* L. has potential in the cosmetic, perfumery or pharmaceutical industries [15] or *Rauvolfia serpentina* L., which presents phytochemical compounds with medicinal value [9]. Some extracts have herbicidal effect [16], or as insecticides [17]. In the pharmaceutical industry the use of extracts have been demonstrated to have antimicrobial activity on gram positive, gram negative bacteria, and fungi [18] [19] [20]. Extracts of *Vateria copallifera bark* have potential for the development of therapeutic agents in the treatment of neurodegenerative diseases [21]. Other extracts with hepatoprotective activity have been found [22].

The potential value in compounds with added value in the different wastes is very high, which makes the study of its composition to have a great interest, as well as scientific, practical. The aim of this work is to contribute to better knowledge of the chemical composition of different lignocellulosic residues generated by agroindustry for the evaluation of the use of them and its main components in other industries and to reduce the effects to the environment.

2. Experimental

2.1. Materials and Reagents

Hexane (HPLC, CALEDON); ethyl ether anhydrous (ACS, MEYER) and acetonitrile (HPLC Plus, SIGMA ALDRICH).

2.2. Sample

Lignocellulosic residues used were obtained from different places in the state of

Morelos; wheat straw (*Triticum aestivum* L.) was provided by the Center for Biological Research of the UAEM; maguey bagasse (*Agave tequilana*) in Miacatlan and sugarcane bagasse (*Saccharum lubridatium*) by Emiliano Zapata sugar mill in Zacatepec. The samples were dried at 105˚C to constant weight, then crushed with a blade mill and stored at room temperature in ziploc bag.

2.3. Extraction

Individual samples of 1.0 g were placed in a 250 ml glass round flask and refluxed for 3 h at 50˚C with 50 ml *n*-hexane. Then organic extracts was concentrated by rotary evaporation at 35˚C under vacuum, and this was further dried under a nitrogen steam. The dry extract was resuspended in 1 ml of *n*-hexane and chromatographed through a silica gel column, which was eluted with *n*-hexane and *n*-hexane-ethyl ether (95: 5) both eluents are combined to form the first fraction (F1). Subsequently, the column was eluted with *n*-hexane-ethyl ether (87:13, 65:35) and ethyl ether, those eluents were also combined to constitute second fraction (F2).

2.4. Derivatization of Second Fraction

The lipophilic extractive (1 mg) of second fraction was silylated with 200 µl BSTFA + TMCS (99:1) in presence of 200 µl pyridine at 70˚C for 1 h before GC-MS analyses. Compounds were identified by comparing their mass spectra with NIST mass spectral database.

2.5. ^{1}H-NMR Spectroscopy

15 mg of the dry extract were weighed and resuspended in $CDCl_3$, the samples were spun down in a micro-centrifuge for 5 min. From supernatant 750 µl were transfered to a 5 mm NMR tube. All spectra were acquired under automation on a Bruker Avance III HD spectrometer operating at 500 MHz.

2.6. GC/MS Analyses

The gas chromatography/mass spectrometry (GC/MS) analyses were performed on a GC equipment (Agilent 6890 instrument) with mass selective detector (Agilent 5973) by electronic impact (70 eV). The separation of the components was performed in a fused silica capillary column (HP-5MS; 30.0 m × 0.25 mm × 0.25 µm). The oven was heated from 40˚C, after 1 min temperature was incremented by 5˚C/min to 285˚C maintaining for 10 min. The injector and transfer line temperatures were set at 250˚C and 300˚C, respectively. Helium was used as the carrier gas and the injection was performed in splitless mode.

3. Results and Discussion

The percentage yield of the total *n*-hexane extracted from dry samples was 1.62 for wheat straw, 1.47 for cane bagasse and 1.64 for maguey bagasse.

The ^{1}H NMR spectrum of the *n*-hexane extract is showed in Figure 1. The most intense signal, occurring a 1.1 ppm, is attributed to methylene aliphatic protons; the signal centered at 0.86 ppm is assigned to methyl protons, which indicates the presence of aliphatic compounds in the majority. Minor signals from additional structural features (protons on carbons adjacent to carbonyl in esters (CH2-C=O) or carboxyl in fatty acids (CH2-COOH) and hydroxyl groups (CHOH), alkene (CH=C) protons) appear at 2.0 - 2.4, 3.2 - 4.1 and 5.1 - 5.5 ppm.

GC/MS Analyses

The result of mass-coupled gas chromatography analysis of the *n*-hexane extracts of fraction 1 (Figure 2) revealed mainly the presence of aliphatic hydrocarbons (saturated and unsaturated) and terpenes (Table 1).

The content of *n*-alkanes in the three samples is between 24.67% - 26.90%, predominantly *n*-alkanes between C25-C29, of these *n*-heptacosane is the most abundant in sugarcane and maguey bagasse; *n*-nonacosane predominates in wheat straw. The *n*-alkanes with fewer carbon atoms (C9-C20) also were found in the samples at an average of 4.5% (Table 1).

Branched alkanes were also found that are rarely in significant amounts in plants (Table 1). As can be observed its content is low, from 1.71% for wheat straw extract to 2.64% for sugarcane bagasse extract. As to its composition can be found differences and similarities; for example, in the three samples are the alkanes whose main chain is 15, 17, 18, 20, 21 and 23 carbon atoms and the methyl group are mainly in the position 3. The alkenes in the samples account for 3.9% and 4.8% of the total content. Overall, the most abundant alkenes are 1-hexadecene, 1-octadecene and 3-eicosene.

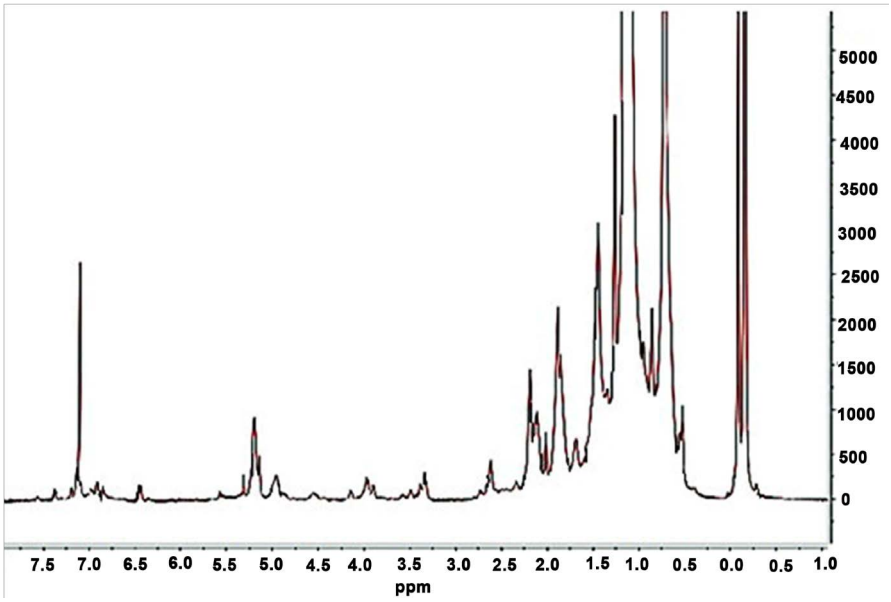

Figure 1. ^{1}H NMR spectrum of the hexane extract of the wheat straw sample.

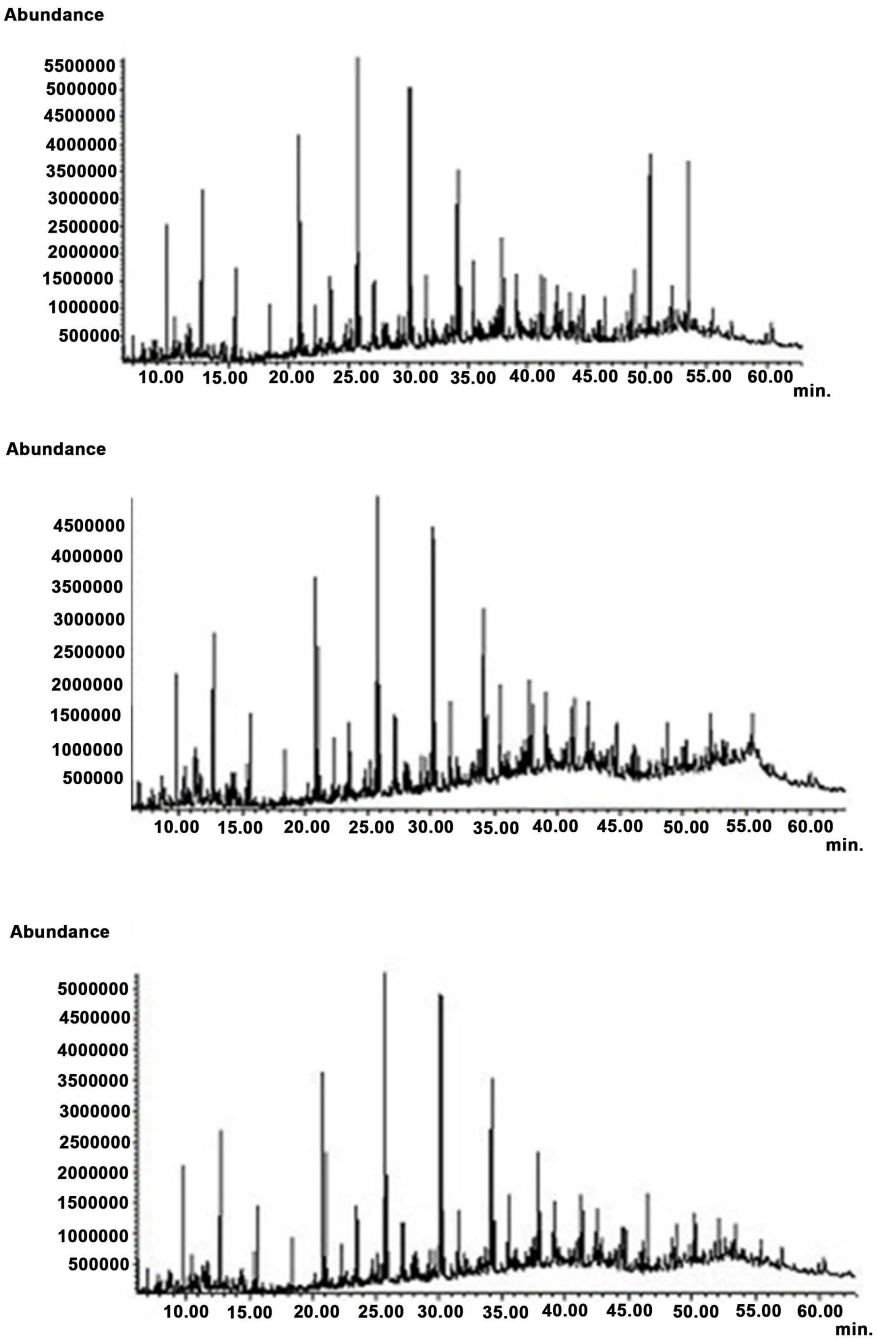

Figure 2. Chromatogram of fraction 1: wheat straw, sugarcane bagasse and maguey bagasse.

The terpenes found ranged from 3.97% of the total wheat straw content to 7.25% in the sugarcane bagasse. The most abundant terpen in the three samples is carveol, account for 50% (**Table 2**). Ketones and fatty alcohols were also identified. The content of ketones in wheat straw and maguey bagasse is 1.64 and 1.51% respectively. However in sugarcane bagasse the content of ketones is 14.74%, due to the abundance of the 14, 16-hentriacontanedione that account for 97.14% of the ketones found in the sugarcane bagasse.

Table 1. Aliphatic hydrocarbons identified in *n*-hexane extracts of agro industrial waste eluted with *n*-hexane and *n*-hexane-ethyl ether (95: 5). (WS: Wheat straw, SB: Sugarcane bagasse, MB: Maguey bagasse, +: present compound, −: no present compound).

Compounds	WS	SB	MB	tr	m/z (abundance)
n-alkanes					
n-nonane	+	+	+	6.95	128(11), 43(100), 57(96.8), 85(44.4)
n-decane	+	+	+	7.86	142(2.4), 57(100), 71(69), 43(47.6)
n-undecane	+	−	+	12.72	156(8.7), 57(100), 43(72.2), 71(54.7)
n-dodecane	+	+	+	15.62	170(7.1), 57(100), 43(70.6), 71(60.3)
n-tridecane	+	+	+	18.39	184(7.1), 57(100), 43(69), 71(63.5)
n-tetradecane	+	+	+	21.04	198(7.1), 57(100), 71(69), 43(64.3)
n-pentadecane	+	+	+	23.52	212(6.3), 57(100), 71(70.6), 43(63.5)
n-hexadecane	+	+	+	25.89	226(7.1), 57(100), 71(72.2), 43(62.7)
n-heptadecane	+	+	+	28.12	240(5.6), 57(100), 71(73.8), 43(60.39
n-octadecane	+	+	+	30.27	254(7.1), 57(100), 71(74.6), 43(61.1)
n-nonadecane	+	+	+	32.22	268 (7.), 57(100), 71(81.2), 43(62)
n-eicosane	+	+	+	34.25	282(5.5), 57(100), 71(77.8), 43(58.7)
n-docosane	−	+	+	37.89	310(4.8), 57(100), 71(82.5), 43(61.1)
n-tricosane	+	+	+	39.42	324(4), 57(100), 71(75.1), 43(61)
n-tetracosane	+	+	+	41.23	338(4.8), 57(100), 71(84.9), 85(61.1)
n-pentacosane	+	+	+	42.80	352(2,4), 57(100), 71(82.5), 85(65.1)
n-hexacosane	+	+	+	44.46	366(3.9), 57(100), 71(78.6), 43(61.1)
n-heptacosane	+	+	+	46.47	380(3.2), 57(100), 71(79.4), 85(63.5)
n-octacosane	+	+	+	48.44	394(4), 57(100), 71(84.9), 85(59.5)
n-nonacosane	+	+	+	50.27	408(4), 57(100), 71(80.2), 85(63.5)
n-triacontane	+	+	+	51.87	422(4), 57(100), 71(77.8), 85(62.8)
n-hentriacontane	−	+	−	53.42	436(4), 57(100), 71(77.2), 85(62.5)
n-dotriacontane	+	+	+	55.46	450(4), 57(100), 71(76.4), 85(62.3)
n-tritriacontane	+	+	−	57.07	464(4.1), 57(100), 71(75.1), 85(62.1)
Branched alkanes					
3-ethyl-2-methyl-heptane	−	+	−	8.06	142(1.6), 57(100), 43(42.9), 98(41.3)
4-methyl-nonane	−	−	+	8.66	142(4), 57(100), 43(62.7), 98(28.6)
2-methyl-nonane	−	−	+	8.73	142(2.4), 57(100), 43(77.8), 71(44.4)
3-methyl-nonane	−	−	+	8.92	142(2.4), 57(100), 71(77.8), 43(43.7)
4-methyl-decane	+	−	+	10.43	156(2.4), 71(100), 57(79.4), 43(77.8)
2-methyl-decane	−	+	+	11.65	156(1.6), 57(100), 43(89.7), 71(72.2)
3-methyl-decane	−	+	+	11.84	156(2.4), 57(100), 71(80.9), 43(57.1)
3-methyl-tetradecane	−	+	−	21.14	212(2.3), 57(100), 43(52.3), 71(49.5)
4-methyl-pentadecane	+	+	+	22.78	226(3.5), 57(87.7), 71(100), 43(91.2)
2-methyl-pentadecane	+	+	−	23.43	226(3.2), 57(100), 71(78.6), 85(69)
3-methyl-pentadecane	+	+	+	25.97	226(2.7), 57(100), 71(53.2), 43(63.1)
2,6,11-tetramethyldodecane	−	+	−	25.19	212(1.6), 43(77), 57(100), 71(65.1)
4-methyl-heptadecane	−	+	−	27.35	254(4.5), 43(100), 71(73.6), 57(66.1)
3-methyl-heptadecane	+	+	+	27.65	254(2.3), 57(100), 71(57.3), 43(50.1)
3-methyl-octadecane	+	+	+	30.36	268(1.3), 57(100), 43(63.9), 41(48.3)
3-methyl-nonadecane	−	+	+	33.69	282(1.1), 57(100),71(46.2), 85(32.2)
2-methyl-eicosane	+	−	+	34.36	296(1), 57(100), 43(87.4), 71(58.9)
3-methyl-eicosane	+	+	+	35.38	296(0.9), 57(100), 43(77.5), 41(49.3)
3-methyl-heneicosane	+	+	+	36.01	310(0.9), 57(100), 43(65.7), 71(55.6)
5-methyl-heneicosane	-	+	-	37.02	310(0.6), 43(100), 57(70.9), 85(56.1)

Continued

2-methyl-tricosane	+	+	+	40.29	338(0.7), 43(100), 57(95.6), 71(48.7)
3-methyl-tricosane	+	+	+	40.62	338(5.4), 57(100), 43(73.1), 71(60.6)
alkenes					
5-methilen-nonane	−	−	+	8.45	140(6.3), 57(100), 69(73.8), 43(65.1)
1-dodeceno	+	+	+	15.37	168(8.2), 41(100), 43(95.2), 55(80.9)
1-tetradecene	+	+	+	20.84	196(8), 43(100), 55(93.3), 57(91)
7-tetradecene	−	+	−	22.24	196(17.9), 55(96.4), 69(100), 43(69.3)
1-hexadecene	+	+	+	25.74	224(7.6), 43(100), 41(87.4), 55(83.1)
1-heptadecene	+	+	−	27.82	238(8.1), 41(100), 43(97.4), 55(86.8)
1-octadecene	+	+	+	30.14	252(7.6), 43(100), 55(96.7), 41(96.5)
3-octadecene	+	+	−	31.53	252(16.7), 69(100), 57(42.1), 55(64.3)
1-nonadecene	+	−	−	32.04	266(18.1), 57(100), 83(98.5), 97(95)
1-eicosene	+	+	+	34.14	280(21.8), 97(100), 57(89.8), 83(86.1)
3-eicosene	−	+	+	35.51	280(19), 57(54.8), 69(100), 55(66.7)
1-docosene	+	+	+	37.79	308(35), 57(100), 43(95.1), 97(86.2)
9-hexacosene	+	−	+	44.38	364(15.1), 43(100), 57(97.3), 97(96.1)

Table 2. Others compounds identified in *n*-hexane extracts of agro industrial waste eluted with *n*-hexane and *n*-hexane-ethyl ether (95: 5). (WS: Wheat straw, SB: Sugarcane bagasse, MB: Maguey bagasse, +: present compound, −: no present compound).

Compounds	WS	SB	MB	tr	m/z (abundance)
Terpenes					
α-farnesene	+	−	+	7.624	204(5.6), 41(100), 93(82.7), 69(62)
limonene	+	+	+	10.786	136(18.1), 68(100), 93(51.2), 67(44.7)
p-cymene	+	+	+	11.496	134(26.1), 119(100), 91(16.6)
carveol	+	+	+	12.291	152(5.3), 119(100), 91(86.1), 134(67.3)
terpinolene	+	+	+	12.462	136(61.2), 93(100), 121(79.1), 91(62)
squalene	+	+	+	48.979	410(1.6), 69(100), 81(57.9), 41(20.6)
Ketones					
4-methyl-2-pentanone	+	−	+	6.78	100(13.9), 43(100), 58(34.5), 41(25.9)
2-methyl-2-penten-4-one	+	+	+	7.84	98(54.3), 83(100), 55(85.1), 43(40.9)
4-hexen-3-one	+	+	+	7.89	98(14.3), 69(100), 41(45.1), 39(14.8)
cyclopentadecanone	+	−	−	20.38	224(11), 55(100), 41(84.7), 71(70.2)
6,10,14-trimethyl-2-pentadecanone	+	+	+	21.65	268(1.7), 43(100), 58(90.1), 71(45.6)
14,16-hentriacontanedione	−	+	−	29.62	464(11.3), 43(100), 100(80.2), 57(75.8)
Alcohols					
1-tetradecanol	+	+	+	22.24	214, 43(100), 55(94.7), 69(74.3)
1-hexadecanol	+	+	+	27.14	242(1.2), 55(100), 69(81.3), 83(77.2)
1-octadecanol	+	+	+	31.53	270(−), 43(100), 83(97.5), 55(94.3)
1-eicosanol	+	+	+	35.52	298(0.5), 43(100), 57(87.1), 55(85.4)
1-docosanol	+	+	+	39.16	326(0.9), 43(100), 57(90.5), 55(89.3)
1-tricosanol	+	+	+	42.50	340(−), 83(100), 57(99.3), 97(93.2)
1-hexacosanol	+	+	+	46.14	382(0.4), 57(100), 43(95.2), 97(80)
1-octacosanol	−	−	+	48.72	410(0.4), 57(100), 43(81.9), 83(79.1)
nonacosanol	+	−	−	50.05	424, 43(100), 57(93.8), 71(84.4)
1-triacontanol	+	+	−	52.53	438(−), 57(85.7), 43(70.6), 82(100)
1-dotriacontanol	−	+	−	53.34	466(−), 68(100), 57(61.9), 82(85.7)
1,30-triacontanediol	−	−	+	56.00	454(−), 57(100), 82(98.4), 69(48.4)

Table 3. Compounds identified in *n*-hexane extracts derivatized of agro industrial waste eluted with *n*-hexane-ethyl ether (87:13, 65:35) and ethyl ether. (WS: Wheat straw, SB: Sugarcane bagasse, MB: Maguey bagasse, +: present compound, −: no present compound).

Compounds	WS	SB	MB	tr	m/z (abundance)
Terpenes					
α-farnesene	+	−	+	7.624	204(5.6), 41(100), 93(82.7), 69(62)
limonene	+	+	+	10.786	136(18.1), 68(100), 93(51.2), 67(44.7)
p-cymene	+	+	+	11.496	134(26.1), 119(100), 91(16.6)
carveol	+	+	+	12.291	152(5.3), 119(100), 91(86.1), 134(67.3)
terpinolene	+	+	+	12.462	136(61.2), 93(100), 121(79.1), 91(62)
squalene	+	+	+	48.979	410(1.6), 69(100), 81(57.9), 41(20.6)
Ketones					
4-methyl-2-pentanone	+	−	+	6.78	100(13.9), 43(100), 58(34.5), 41(25.9)
2-methyl-2-penten-4-one	+	+	+	7.84	98(54.3), 83(100), 55(85.1), 43(40.9)
4-hexen-3-one	+	+	+	7.89	98(14.3), 69(100), 41(45.1), 39(14.8)
cyclopentadecanone	+	−	−	20.38	224(11), 55(100), 41(84.7), 71(70.2)
6,10,14-trimethyl-2-pentadecanone	+	+	+	21.65	268(1.7), 43(100), 58(90.1), 71(45.6)
14,16-hentriacontanedione	−	+	−	29.62	464(11.3), 43(100), 100(80.2), 57(75.8)
Alcohols					
1-tetradecanol	+	+	+	22.24	214, 43(100), 55(94.7), 69(74.3)
1-hexadecanol	+	+	+	27.14	242(1.2), 55(100), 69(81.3), 83(77.2)
1-octadecanol	+	+	+	31.53	270(−), 43(100), 83(97.5), 55(94.3)
1-eicosanol	+	+	+	35.52	298(0.5), 43(100), 57(87.1), 55(85.4)
1-docosanol	+	+	+	39.16	326(0.9), 43(100), 57(90.5), 55(89.3)
1-tricosanol	+	+	+	42.50	340(−), 83(100), 57(99.3), 97(93.2)
1-hexacosanol	+	+	+	46.14	382(0.4), 57(100), 43(95.2), 97(80)
1-octacosanol	−	−	+	48.72	410(0.4), 57(100), 43(81.9), 83(79.1)
nonacosanol	+	−	−	50.05	424, 43(100), 57(93.8), 71(84.4)
1-triacontanol	+	+	−	52.53	438(−), 57(85.7), 43(70.6), 82(100)
1-dotriacontanol	−	+	−	53.34	466(−), 68(100), 57(61.9), 82(85.7)
1,30-triacontanediol	−	−	+	56.00	454(−), 57(100), 82(98.4), 69(48.4)

The alcohols identified represent 7.18% and 7.38% of the extract of the wheat straw and the cane bagasse respectively, while the maguey bagasse is 9.09%. Identifying the presence of alcohols from C14-C32, being found in the extracts of the three residues up to *n*-hexacosanol. The 1, 30-triacontanediol was only observed in the maguey bagasse extract.

The fraction 2 was derivatized with the aim of increasing the volatility of the compounds and thus obtaining a greater intensity in the chromatogram of the samples analyzed (**Figure 3**).

In the chromatogram of the derivatized extract it was possible to identify several fatty acids, most of which are found in the sugarcane and maguey bagasse, only three acids could be identified in the extract of the wheat straw. Also in these extracts could be identified several steroidal compounds, predominating

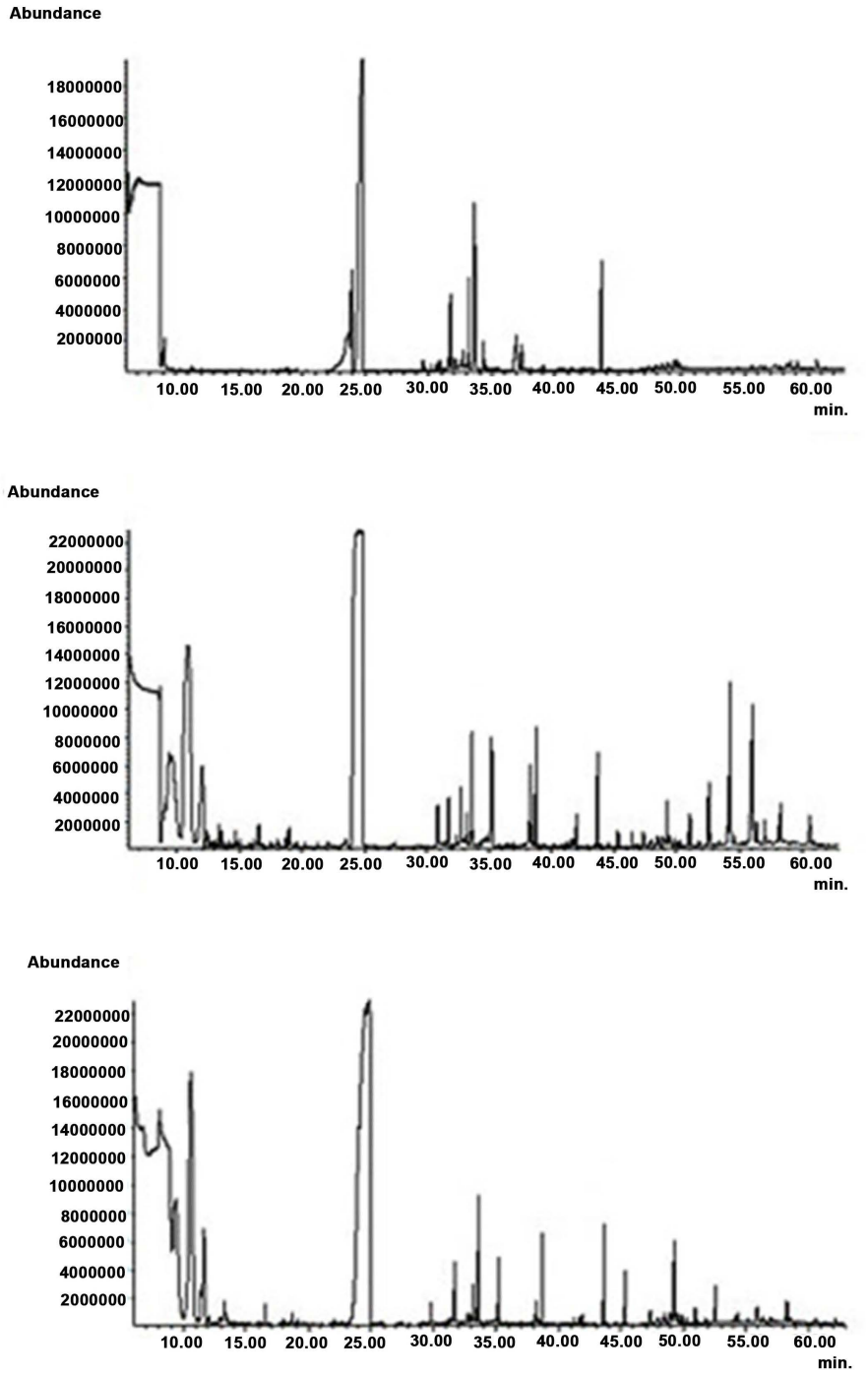

Figure 3. Chromatogram of second fraction derived: wheat straw, sugarcane bagasse and maguey bagasse.

the sterols in the extracts of maguey and sugarcane bagasse, whereas in the wheat straw steroids ketones predominate (**Table 3**).

4. Conclusion

In conclusion, the present paper provides a comprehensive description of the lipophilic compounds in *n*-hexane extracts in three lignocellulosic wastes, which

is highly valuable information for a more complete industrial utilization of these lignocellulosic materials.

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