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Lipophilic extractives from different morphological parts of banana plant "Dwarf Cavendish"

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Abstract

The chemical composition of the dichloromethane extracts of different morphological parts of banana plant "Dwarf Cavendish", cultivated in Madeira Island (Portugal), were studied by gas chromatography–mass spectrometry. The five different morphological fractions in study have a similar qualitative chemical composition. Fatty acids and sterols are the major families present in the lipophilic extract of "Dwarf Cavendish", representing ca. 33–66% and 12–43%, respectively, of the total amount of lipophilic components. Among all the identified compounds, campesterol, stigmasterol, sitosterol and fatty acids, such as palmitic, stearic, linoleic, linolenic, 22-hydroxydocosanoic, 24-hydroxytetracosanoic and 26-hydroxyhexacosanoic acids, were the major components found in all morphological zones. Other families of compounds, such as aromatic compounds, fatty alcohols and alkanes were also identified. The high increase of some components after alkaline hydrolysis, particularly, ferulic and fatty acids, indicates the presence of a considerable fraction of such components in esterified structures.

Keywords: Banana plant; Dwarf Cavendish; Fatty acids; GC–MS analysis; Lipophilic extractives; *Musa acuminata* Colla; Sterols

1. Introduction

Banana plant is a very important crop in the Madeira Island. According to official statistics, the banana plantations produce annually ca. 30,000 tonnes of fruit representing a substantial economic profit to this region. However, after collecting the single bunch of bananas, great amounts of agricultural residues are produced. These residues, find little or no use apart from being used as organic material in plantations.

"Dwarf Cavendish" (*Musa acuminata* Colla var *cavendish*) is presently the most important cultivar, representing ca. 50–60% of the total banana production in

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Madeira Island. The agricultural residues produced on the plantations reach around 15,000 tonnes/year.

The development of new applications for these agricultural residues together with rachis (a by-product of banana processing), as a non-wood fibre source, might be an important contribution to the valorisation of banana plantations in Madeira Island, as well as in other worldwide regions, where banana plantations have an important economic impact.

The use of banana plant pseudo-stems and rachis as a potential source of fibres for pulp production and papermaking has been studied for various species (Cordeiro et al., 2004 and references therein). Preliminary studies of pulping of *M. acuminata* Colla residues show that the polysaccharides and lignin contents encourage the use of this species as a suitable source of fibre (Cordeiro et al., 2004). The high extractives content found (ca. 7–19%) was considered as a detrimental aspect as far as fibre production is concerned, but it can also be considered as an alternative source of valorisation of banana plant residues.

As part of a research project aiming to find new applications for the banana plants vegetal residues produced after the harvesting of fruits, we have been studying the chemical composition of the plant tissues from different morphological regions of "Dwarf Cavendish" (*M. acuminata* Colla var *cavendish)*, from Madeira Island. In a previous communication (Oliveira et al., 2005), we have reported the detection of an abundant fraction of steryl glucosides in "Dwarf Cavendish" dichloromethane extracts. In that preliminary study (Oliveira et al., 2005), it became evident that a complex mixture of aliphatic components composed such extracts.

The exhaustive identification of such fraction was, therefore, required within the scope of the present project, and especially, when it is known that the bibliography covering banana plant extractives is quite scarce. Published results deal mainly with the composition of banana peel and pulp from other *Musa* species (Knapp and Nicholas, 1969a,b,c, 1971; Knapp et al., 1972; Ghosal and Saini, 1984; Ghosal, 1985; Akihisa et al., 1986), and more recently, the composition of the volatile fraction of banana cultivars, including "Dwarf Cavendish, from Madeira Island has been reported (Nogueira et al., 2003), however, no chemical characterisation of the other extractives fractions of "Dwarf Cavendish" has been published so far.

The present paper aims to describe a detailed study of the composition of "Dwarf Cavendish" lipophilic extractives (in dichloromethane) by gas chromatography–mass spectrometry (GC–MS), before and after alkaline hydrolysis, in order to analyse all free and esterified components.

2. Materials and methods

2.1. Samples

Mature plants of "Dwarf Cavendish", were randomly selected from a plantation in Funchal (Madeira Island). Plants were harvested and fractionated into five different morphological parts. The pseudo-stems were handly separated into leaf sheaths and floral stalk. Foliage was also separated in petioles/midrib and leaf blades. Rachis was collected in a Banana Cooperative. After separation, the samples were air-dried during 2 weeks. All morphological parts were milled and sieved, and the fraction of 40–60-mesh was selected.

2.2. Extraction

The powdered samples (2 g × 20 g) from the different morphological parts of "Dwarf Cavendish" were Soxhlet extracted with dichloromethane for 6 h. The solvent was evaporated to dryness and the extracts were weighed.

2.3. Alkaline hydrolysis

About 20 mg of each extract were dissolved in 10 ml of 1 M KOH in 10% aqueous methanol. The mixtures were heated at 100 °C, under nitrogen, during 1 h. The reaction mixtures were cooled, acidified with 1 M HCl to pH ∼ 2 and then extracted three times with dichloromethane. The solvent was evaporated to dryness.

2.4. GC–MS analysis

Before GC–MS analysis, nearly, 20 mg of each dried sample (extracts and hydrolysed extracts), with a measured amount of internal standard, were dissolved in 250 μl of pyridine and the compounds containing hydroxyl and carboxyl groups were converted into

trimethylsilyl (TMS) ethers and esters, respectively, by adding 250 μl of bis(trimethylsilyl)trifluoroacetamide and 50 μl of trimethylchlorosilane. After the mixture had stood at 70 °C for 30 min, the derivatized extracts were analysed by GC–MS (Freire et al., 2002a,b). In order to confirm the identification of some sterols as acetylated derivatives, an aliquot of the dichloromethane extract of leaf sheaths was treated with acetic anhydride, in pyridine, at 100 °C for 1 h, the sample was then submitted to trimethylsilylation as described above and analysed by GC–MS.

GC–MS analysis were performed using a trace gas chromatograph 2000 Series equipped with a Finnigan Trace MS mass spectrometer, using helium as carrier gas (35 cm/s), equipped with a DB-1 J&W capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature rate, 4 °C/min; final temperature, 285 °C for 10 min; injector temperature, 290 °C; transfer-line temperature, 290 °C; split ratio, 1/100.

In order to verify the presence of esterified structures, such as triglycerides, steryl esters, waxes and ferulic acid esters, the extracts were also analysed by GC–MS using a DB-1 J&W capillary column (15 m × 0.32 mm i.d., 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature, 100 °C for 3 min; temperature rate, 5 °C/min; final temperature, 340 °C for 12 min; injector temperature, 320 °C; transfer-line temperature, 290 °C; split ratio, 1/100.

Components were identified based on the comparison of their spectra with a spectral library (Wiley-Nist), based on retention times obtained under the described experimental conditions (Freire et al., 2002a,b) and, in some cases (see Section 3) by comparing their fragmentation profiles with published data.

For quantitative analysis, GC–MS was calibrated with pure reference compounds, representative of the major lipophilic extractives components (namely hexadecanoic acid, 1-eicosanol, 16-hydroxyhexadecanoic acid, 2-hydroxyoctadecanoic acid, stigmasterol and ferulic acid), relative to tetracosane, the internal standard used. The respective response factors were calculated as an average of six GC–MS runs.

Two aliquots of each extract were analysed before alkaline hydrolysis and another two after hydrolysis. Each aliquot was injected in triplicate. The presented results are the average of the values obtained for each part.

2.5. Chemicals

The 16-hydroxyhexadecanoic acid (97% purity) and 1-eicosanol (98% purity) were purchased from Fluka Chemie (Madrid, Spain); stigmasterol (95% purity), hexadecanoic acid (99% purity), tetracosane (99% purity), ferulic acid (99% purity), acetic anhydride (99% purity), pyridine (99% purity) bis(trimethylsilyl)trifluoroacetamide (99% purity) and trimethylchlorosilane (99% purity) were supplied by Sigma Chemicals Co. (Madrid, Spain). The 2-hydroxyoctadecanoic acid was kindly provided by Dr. Les West from Kraft Foods, USA.

3. Results and discussion

The yields of dichloromethane extractives from the different morphological parts of “Dwarf Cavendish” are quite similar, except for leaf blades. Petioles/midrib, floral stalk, leaf sheaths and rachis extracts account for 1.2, 1.4, 1.4 and 1.5% of dry material, respectively, whereas the value obtained for leaf blades is much higher (5.8%). The values found for petioles/midrib, floral stalk, leaf sheaths and rachis are similar to those reported for other annual plants, such as rice straw (Xiao et al., 2001) and wheat straw (Sun and Sun, 2001).

The qualitative composition of the dichloromethane extracts of the several parts of “Dwarf Cavendish” is quite similar, however, the abundance of certain compounds differs significantly. Fig. 1 shows a typical chromatogram of leaf sheaths derivatized extracts after alkaline hydrolysis. Tables 1–4 list the identified families and individual components and their corresponding abundances in the dichloromethane extracts of the several morphological parts, before and after alkaline hydrolysis.

Fatty acids and sterols are the major families of the lipophilic components found in the different morphological parts of this banana plant species (Fig. 2). Apart from the above-referred families, minor amounts of aromatic compounds and aliphatic alcohols, among others, were also identified (Fig. 2). After alkaline hydrolysis, a large increase in the total amount of

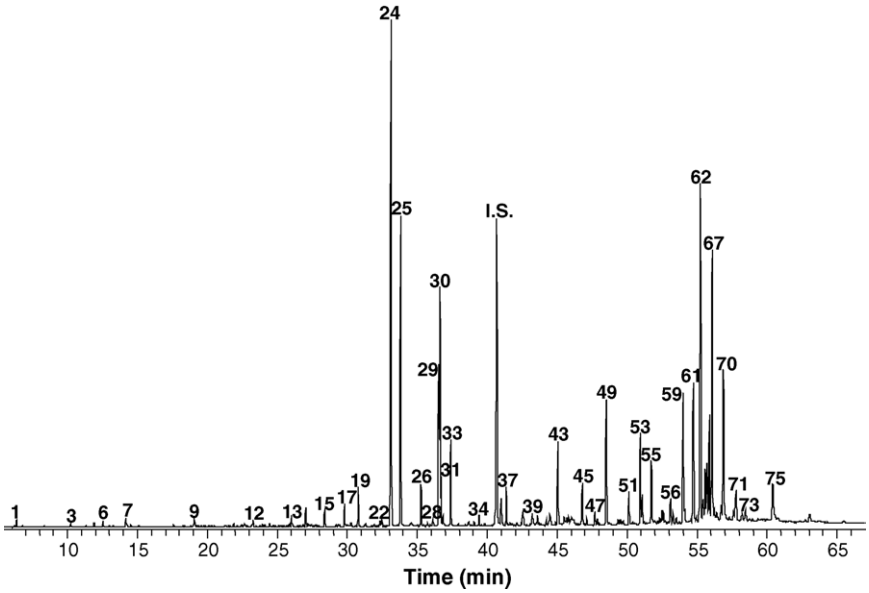

Fig. 1. Total ion chromatogram of the derivatized dichloromethane extract of leaf sheaths of "Dwarf Cavendish" after alkaline hydrolysis (AH). I.S.: internal standard. Peaks identification in Tables 1–4 (peaks whose identification is not present in Tables 1–4 correspond to sililation artefacts and solvent impurities).

extractives detected by GC–MS was observed, particularly, among the fatty acids (Fig. 2). This suggests the presence of significant amounts of esterified structures in the original extracts, as will be discussed below.

3.1. Fatty acids composition

Before alkaline hydrolysis, saturated fatty acids, such as hexadecanoic, octadecanoic and tetracosanoic acids are the most abundant components of this family in all morphological parts, ranging from 53 to 88% of the total fatty acids amount (Table 1). After alkaline hydrolysis, saturated fatty acids are still the most abundant group (except in rachis) with increases ranging from 17% (floral stalk) up to 130% (petioles/midrib). As before alkaline hydrolysis, hexadecanoic, octadecanoic and tetracosanoic acids are the major compounds of this group (Table 1).

Odd-numbered chain fatty acids, namely nonanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, pentacosanoic and heptacosanoic acids were also identified (Table 1). These compounds are quite common in annual plants, such as rice straw (Xiao et al., 2001) and wheat straw (Sun and Sun, 2001).

Unsaturated fatty acids are also present, representing in the case of floral stalk ca. 43% of the total fatty acids analysed before alkaline hydrolysis. Linoleic, linolenic and oleic acids are the most abundant compounds of this group (Table 1). After alkaline hydrolysis, an increase in the amount of unsaturated fatty acids (particularly, in linoleic and linolenic acids) was observed in most of the morphological parts. This increase was particularly, significative in rachis (704%) (Table 1).

Several α- and ω-hydroxy fatty acids were identified in the dichloromethane extracts of the banana plant, representing between 1 and 8% of the total fatty acids, before alkaline hydrolysis. ω-Hydroxyfatty acids are the most abundant hydroxyfatty acids found in most of the morphological parts, particularly, after alkaline hydrolysis of the extracts (Table 1) with increases ranging between 52% (rachis) to 1700% (petioles/midrib). The 22-hydroxydocosanoic, 24-hydroxytetracosanoic and 26-hydroxyhexacosanoic acids are the major components of this group. After alkaline hydrolysis, the amounts of these components decreased slightly in

Table 1
Fatty acid components (mg of compound/kg of dry material) identified in the dichloromethane extracts of different morphological parts of "Dwarf Cavendish" before (BH) and after (AH) alkaline hydrolysis

Peak	Compound	Petioles/midrib		Leaf blades		Floral stalk		Leaf sheaths		Rachis	
		BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	Fatty acids	991	2775	6970	9623	4909	6268	2793	4345	2955	5309
	Saturated	770	1770	5841	8046	2609	3135	1809	2417	2611	4111
4	Octanoic acid	tr	4	43	6	2	3	2	3	7	4
8	Nonanoic acid	tr	3	tr	n.d.	tr	tr	tr	3	tr	3
12	Dodecanoic acid	4	17	24	112	8	16	6	11	22	39
15	Tetradecanoic acid	11	8	220	231	47	63	20	31	64	98
19	Pentadecanoic acid	22	58	119	146	99	124	54	72	53	146
24	Hexadecanoic acid	444	810	2356	3612	1578	1775	849	1173	1515	2451
26	Heptadecanoic acid	24	60	114	211	68	76	60	79	58	88
33	Octadecanoic acid	55	126	332	508	168	215	105	168	188	188
34	Nonadecanoic acid	5	15	18	39	11	14	16	21	20	27
37	Eicosanoic acid	22	57	278	330	74	88	50	70	61	99
39	Heneicosanoic acid	34	13	120	77	19	18	33	17	31	22
43	Docosanic acid	32	160	583	939	139	193	115	161	100	231
45	Tricosanoic acid	23	57	179	147	57	70	78	85	68	97
49	Tetracosanoic acid	58	215	507	769	198	282	199	271	175	304
51	Pentacosanoic acid	14	42	244	83	61	77	62	70	67	88
55	Hexacosanoic acid	12	83	175	270	63	107	77	131	65	105
57	Heptacosanoic acid	tr	10	78	26	17	14	34	tr	35	20
71	Triacontanoic acid	10	32	451	540	tr	tr	49	51	82	101
	Unsaturated	157	388	142	113	2096	2316	618	901	113	908
22	9-Hexadecenoic acid (*cis* or *trans*)	tr	8	tr	tr	87	10	8	9	tr	tr
23	9-Hexadecenoic acid (*cis* or *trans*)	tr	8	n.d.	n.d.	84	101	8	8	24	80
29	9,12-Octadecadienoic acid	49	122	27	n.d.	1060	1206	245	360	33	306
30	9,12,15-Octadecatrienoic acid	66	174	28	n.d.	699	844	291	429	17	287
31	9-Octadecenoic acid (*cis* or *trans*)	35	68	87	113	81	79	53	75	12	159
32	9-Octadecenoic acid (*cis* or *trans*)	7	8	n.d.	n.d.	85	76	13	20	27	76
	Diacids	8	65	230	413	7	28	12	52	37	65
14	Nonadioic acid	8	47	230	356	7	28	12	32	37	59
21	Undecanedioic acid	n.d.	4	tr	57	tr	n.d.	n.d.	tr	tr	6
72	Hexacosanedioic acid	n.d.	14	n.d.	n.d.	n.d.	n.d.	n.d.	20	n.d.	tr
	α-Hydroxy acids	29	67	333	51	49	28	132	108	81	53
42	2-Hydroxyeicosanoic acid	13	tr	57	9	18	5	17	8	28	9
48	2-Hydroxydocosanoic acid	tr	13	tr	13	tr	tr	15	12	11	tr
54	2-Hydroxytetracosanoic acid	16	42	276	29	15	15	72	63	31	29
60	2-Hydroxyhexacosanoic acid	n.d.	12	tr	tr	16	8	28	25	11	15
	ω-Hydroxy acids	27	485	424	1000	148	761	222	867	113	172
41	18-Hydroxyoctadecanoic acid	n.d.	3	n.d.	tr	n.d.	9	n.d.	11	n.d.	n.d.
47	20-Hydroxyeicosanoic acid	n.d.	12	n.d.	14	n.d.	26	tr	26	tr	tr
53	22-Hydroxydocosanoic acid	6	132	374	699	23	250	37	209	32	51
59	24-Hydroxytetracosanoic acid	tr	152	tr	213	18	290	32	263	7	27
63	25-Hydroxypentacosanoic acid	n.d.	19	tr	tr	n.d.	13	12	37	tr	16
70	26-Hydroxyhexacosanoic acid	21	83	50	74	107	142	88	187	74	78
73	27-Hydroxyheptacosanoic acid	n.d.	14	n.d.	n.d.	n.d.	n.d.	n.d.	28	n.d.	tr
75	28-Hydroxyoctacosanoic acid	n.d.	70	tr	tr	tr	31	53	106	n.d.	tr

tr: traces; n.d.: non-detected.

Table 2
Triterpene components (mg of compound/kg of dry material) identified in the dichloromethane extracts of different morphological parts of "Dwarf Cavendish", before (BH) and after (AH) alkaline hydrolysis

Peak	Compound	Petioles/midrib		Leaf blades		Floral stalk		Leaf sheaths		Rachis	
		BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	Sterols	1269	1306	2436	1921	2372	2462	2205	2570	2003	2552
56	Cholesterol + α-tocopherol[a]	48	45	263	112	69	64	53	51	54	39
61	Campesterol + octacosanoic acid[a]	186	222	609	574	342	327	338	393	312	427
62	Stigmasterol	357	323	417	343	939	1020	712	869	696	975
64	24-Methylenepollinastanol	36	37	167	76	n.d.	86	88	95	54	76
65	31-Norcyclolaudenone	13	17	n.d.	n.d.	74	41	117	99	168	154
66	Cycloeucalenone	66	66	n.d.	n.d.	100	79	217	203	325	340
67	β-Sitosterol	532	481	930	742	639	613	520	605	281	423
69	Cycloeucalenol	n.d.	n.d.	n.d.	n.d.	15	16	23	17	39	40
70	Cycloartenol	21	83	50	74	107	142	88	187	74	78
71	24-Methylenecycloartanol	10	32	n.d.	n.d.	87	74	49	51	tr	tr

tr: traces; n.d.: non-detected.
[a] Co-elution of cholesterol with α-tocopherol and campesterol with octacosanoic acid. Cholesterol and campesterol are the major components in almost of the morphological parts.

almost of the morphological regions (except for petioles/midrib), with the higher variation observed in leaf blades. This decrease can be explained, by the known tendency of these compounds to decarboxylate under alkaline conditions, yielding the corresponding saturated fatty acids with one less carbon atom (Freire et al., 2003). On other hand, α-hydroxy fatty acids appear in minor amounts. The 2-hydroxyeicosanoic and 2-hydroxytetracosanoic are the major α-hydroxyacids found in most of the morphological parts (Table 1). The presence of α-hydroxyfatty acids has already been reported in some other herbaceous plants, such as *Thymus vulgaris* L. (Smith and Wolff, 1969) and *Arabidopsis thaliana* (Imai et al., 2000).

Minor amounts of diacids, such as nonadioic acid were also found in all extracts (Table 1). The amount of these acids also increased substantially after alkaline hydrolysis.

The increase in fatty acids after hydrolysis is mainly due to components with more than 16 carbon atoms. In leaf blades and rachis the increase is mainly due to acids in the range from C-16 to C-20, whereas in

Table 3
Long-chain aliphatic alcohols components (mg of compound/kg of dry material) identified in the dichloromethane extracts of different morphological parts of "Dwarf Cavendish", before (BH) and after (AH) alkaline hydrolysis

Peak	Compound	Petioles/midrib		Leaf blades		Floral stalk		Leaf sheaths		Rachis	
		BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	Long-chain aliphatic alcohols	39	253	1034	1836	29	90	40	60	57	301
20	1-Hexadecanol	tr	6	tr	34	4	10	tr	4	tr	10
27	1-Octadecanol	4	10	tr	18	tr	9	tr	6	tr	9
35	1-Eicosanol	tr	6	tr	19	tr	6	tr	tr	tr	tr
40	1-Docosanol	tr	11	25	47	tr	31	6	15	tr	20
46	1-Tetracosanol	tr	17	n.d.	45	3	12	4	12	tr	37
52	1-Hexacosanol	tr	24	130	122	n.d.	4	tr	5	16	98
58	1-Octacosanol	12	54	250	343	15	10	15	10	16	86
68	1-Triacontanol	23	112	538	1019	7	8	15	8	25	41
74	1-Dotriacontanol	n.d.	13	91	189	n.d.	n.d.	n.d.	n.d.	n.d.	tr

tr: traces; n.d.: non-detected.

Table 4
Aromatic components (mg of compound/kg of dry material) identified in the dichloromethane extracts of different morphological parts of "Dwarf Cavendish", before (BH) and after (BH) alkaline hydrolysis

Peak	Compound	Petioles/midrib		Leaf blades		Floral stalk		Leaf sheaths		Rachis	
		BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	Aromatic compounds	51	365	887	435	24	420	128	732	28	92
3	Benzoic acid	tr	2	26	20	tr	n.d.	5	5	10	9
7	*p*-Hydroxybenzaldehyde	n.d.	3	n.d.	n.d.	2	tr	23	25	tr	15
9	Vanillin	tr	30	103	13	12	8	20	18	11	14
11	*p*-Hydroxybenzoic acid	4	2	n.d.	n.d.	n.d.	n.d.	26	5	tr	n.d.
13	Vanillic acid	22	36	141	97	2	3	18	18	7	9
16	Siringic acid	7	17	tr	52	n.d.	tr	tr	3	tr	5
17	*cis*-Ferulic acid	n.d.	17	185	31	n.d.	58	n.d.	41	n.d.	5
18	*p*-Coumaric acid	6	14	334	88	n.d.	n.d.	tr	5	n.d.	tr
25	*trans*-Ferulic acid	12	244	98	134	8	351	36	612	tr	35

tr: traces; n.d.: non-detected.

floral stalk and leaf sheaths is mainly due to acids higher than C-20. The high increase observed in the amounts of fatty acids after alkaline hydrolysis, indicates that an important fraction of these compounds is present in the plant in esterified forms. In order to check the presence of fatty acid esters in banana plant, the dichloromethane extracts (before hydrolysis) were analysed by GC–MS with a 15 m column, using chromatographic conditions, which allow the elution and detection of such low-volatile lipophilic compounds (Freire et al., 2002a,b). Six monoglycerides, namely 1-monohexacosanoylglycerol, 1-monooctacosanoylglycerol, 1-monotriacontanoylglycerol, 1-mono(24-hydroxytetracosanoyl)glycerol, 1-mono(26-hydroxyhexacosanoyl)glycerol and 1-mono(28-hydroxyoctacosanoic)glycerol, were identified in the various morphological parts, based on their relative retention times and on their fragmentation patterns (Graça and Pereira, 2000). Steryl esters, such as cycloartenyl octadecanoate (or cycloeucalenyl octadecanoate) and 24-methylenecycloartenyl octadecanoate were also identified, based on their characteristic fragmentations and

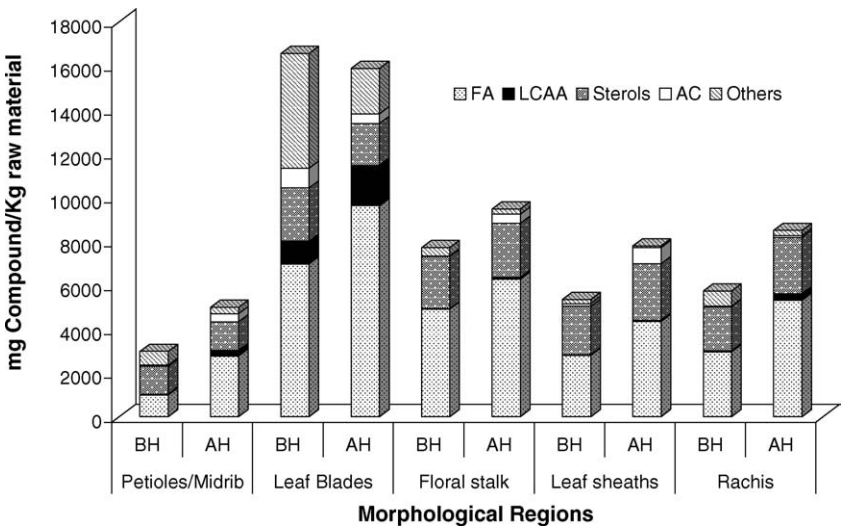

Fig. 2. Major families of compounds identified in the dichloromethane extracts of "Dwarf Cavendish" before (BH) and after (AH) alkaline hydrolysis. FA: fatty acids; LCAA: long-chain aliphatic alcohols; ST: sterols; AC: aromatic compounds.

retention times, in all morphological parts. However, the small amounts of glycerides and steryl esters of fatty acids detected, in the GC–MS analysis, cannot explain the increase in the amount of fatty acids observed after alkaline hydrolysis. This means that fatty acids are present in other esterified structures. On the other hand, this increase cannot also be explained by the presence of wax esters, since these structures were not detected by GC–MS, which is consistent with the small increase of aliphatic alcohols observed after alkaline hydrolysis (see Section 4). A possible explanation is the presence of such fatty acids esterified with non-volatile high molecular weight suberin or cutin type structures, not detected by GC–MS (Cordeiro et al., 1998; Lopes et al., 2000).

Although the presence of hexadecanoic and oleic acids, particularly, in esterified forms, has already been discussed in other *Musa* species (Knapp and Nicholas, 1969a,b; Ghosal and Saini, 1984; Ghosal, 1985), to our knowledge, a detailed identification and quantification of fatty acids in banana plant, as described here, has not been reported so far.

3.2. Sterols composition

Sterols identified in "Dwarf Cavendish" (Fig. 3) represent 12–13% of the lipophilic components (Fig. 2). β-Sitosterol followed by campesterol and stigmasterol are the major sterols present in all morphological parts (Table 2). Peak **64** was firstly identified as 24-methylenepollinastanol based on the detection of the fragments at *m*/*z* 484 $[M]^+$, 469 $[M–CH_3]$, 441 $[M–C_3H_7]$, 394 [–TMS], 379 $[M–CH_3–TMS]$ and 351 [441-TMSOH]. This identification was confirmed by the GC–MS analysis of the acetylated sample, based on the detection of characteristic fragments of 24-methylenepollinastanyl acetate, *m*/*z* 454 $[M]^+$, 439 $[M–CH_3]$, 411 $[M–C_3H_7]$, 394 [M–HOAc], 379 $[M–CH_3–HOAc]$, 351 [411-HOAc], 329 [M–side chain (C_9H_{17})] (Akihisa et al., 1986).

The mass spectra of peaks **65** and **66** show a very similar mass fragmentation pattern, characteristic of triterpene derivatives. The absence of the fragment at *m*/*z* 73, (TMS) indicates that these compounds do not have any free hydroxyl group. However, their molecular ion at *m*/*z* 424 is consistent with a molecular formula of $C_{30}H_{48}O$ of a triterpenic ketone. The mass spectra, with fragments at *m*/*z* 424, 409 $[M–CH_3]$; 381 $[M–C_3H_7]$, 367, 355 $[M–C_5H_{10}]$, 341 $[M–C_6H_{11}]$, 327 $[M–C_7H_{13}]$ and 299 $[M–C_9H_{17}]$ are characteristic of two triterpene ketones previously identified in banana peel and in flowers of some *Musa* species,

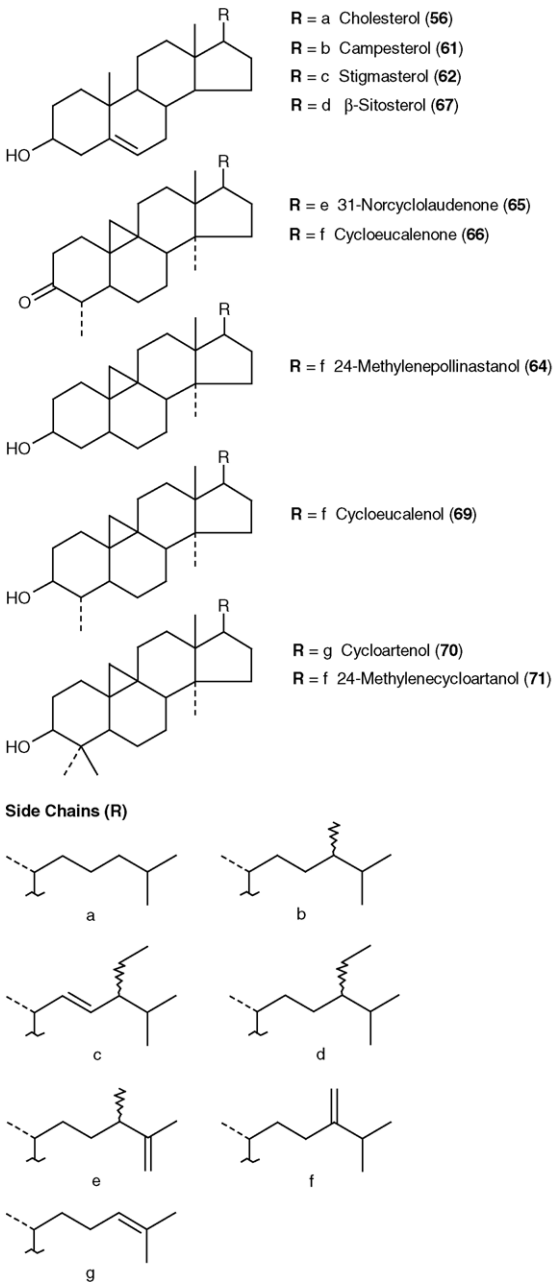

Fig. 3. Structures of the sterols identified in banana plant "Dwarf Cavendish".

cycloeucalenone and 31-norcyclolaudenone (Knapp and Nicholas, 1969b; Banerji et al., 1982; Akihisa et al., 1986). The distinction of these two isomeric ketones was only achieved based on the fact that cycloeucalenone elutes later than 31-norcyclolaudenone and also on the fact that the fragment at *m*/*z* 341 is more intense in cycloeucalenone due to the unsaturation at C24 (Akihisa et al., 1998).

Chromatographic peaks **69** and **70** also present similar fragmentation patterns, the most relevant peaks in their mass spectra are *m*/*z* 498 $[M]^+$, 483, $[M–CH_3]$, 408 [M–TMSOH], 393 $[M–TMSOH–CH_3]$, 365, 353, 300 and 283. This fragmentation pattern is characteristic of the TMS derivatives of cycloeucalenol and cycloartenol (Fig. 3) previously reported (Knapp and Nicholas, 1969b); the identification of peaks **69** and **70** as cycloeucalenol and cycloartenol, respectively, is based on their known elution order (Knapp and Nicholas, 1969b; Akihisa et al., 1986).

Peaks **56** and **71** were identified as cholesterol and 24-methylenecycloartanol, respectively, based on the characteristic fragmentations of their TMS derivatives (Niemelä, 1990; Xu and Godber, 1999); the identification of the former was also confirmed by injection of a reference sample.

After alkaline hydrolysis, the relative proportion of the different sterols remain roughly unchanged, which means that the proportions of free sterols and of esterified sterols are very similar in banana plant. The small increase in the amounts of sterols observed after alkaline hydrolysis are in agreement with the moderate amounts of steryl ester detected in the GC–MS analysis with 15 m column, as referred above. Finally, it is also worth to mention that the variation in the amounts of sterols with hydrolysis is not related to the presence of the previously reported high amounts of steryl glucosides (Oliveira et al., 2005), since these acetal type structures are resistant to alkaline hydrolysis.

The major sterols identified in “Dwarf Cavendish” have already been reported in banana pulp and peel of other *Musa* species (Knapp and Nicholas, 1969a,b,c, 1971; Knapp et al., 1972; Banerji et al., 1982; Dutta et al., 1983; Ghosal and Saini, 1984; Ghosal, 1985; Akihisa et al., 1986, 1998). Campesterol, stigmasterol, sitosterol and cycloeucalenol have already been identified in the stalk, rhizome and leaves of *Musa sapentium*, while cycloartenol and 24-methylenecycloartanol have been identified in its stalk and rhizome (Knapp and Nicholas, 1969a).

3.3. Fatty alcohols composition

Fatty alcohols represent only a small fraction of the total amount of lipophilic extractives analysed by GC–MS, except in leaf blades, where higher amounts were detected (Fig. 2). The most abundant fatty alcohols found in “Dwarf Cavendish” are those higher than C-20, namely 1-octacosanol and 1-triacontanol followed by 1-docosanol and 1-tetracosanol (Table 3). The amounts of these compounds also increase moderately after alkaline hydrolysis. The presence of fatty alcohols in “Dwarf Cavendish” is reported here for the first time.

3.4. Aromatic and other components

Before alkaline hydrolysis, leaf blades present the highest content of aromatic compounds (Fig. 2 and Table 4), whereas in floral stalk and rachis, aromatic compounds are present in smaller amounts. In leaf blades, coumaric acid followed by ferulic acid represent 75% of the aromatic fraction. Minor amounts of *p*-hydroxybenzaldehyde, vanillin and benzoic, *p*-hydroxybenzoic, vanillic and syringic acids, were also detected in the several parts of banana plant (Table 4). After alkaline hydrolysis, the increase in the amount of aromatic compounds is mainly due to ferulic acid. This compound is present in considerable amounts in leaf sheaths and floral stalk, whereas in rachis, it is identified in smaller amounts (Table 4). Apart from the occurrence of ferulic acid esterified with lignin and polysaccharides in herbaceous plants (Ralph and Helm, 1993), ferulic acid can be found esterified with other kind of compounds, such as fatty alcohols in several woods (Kolattukudy and Espelie, 1989; Ekman and Holmbom, 1989) and with ω-hydroxyfatty acids (Kolattukudy and Espelie, 1989; Cordeiro et al., 1998; Lopes et al., 2000). The analysis of the extract with a 15 m column allowed the detection of trace amounts of two ferulates, namely docosanyl and tetracosanyl ferulates, in petioles/midrib, floral stalk and leaf sheaths. In this way, the amounts of ferulic acid detected after hydrolysis cannot be explained based on the amounts of these derivatives; implying that, ferulic acid should be esterified with other compounds. Once more, a pos-

sible explanation for the origin of ferulic acid in banana plant is its esterification with higher molecular weight suberin or cutin type structures (Cordeiro et al., 1998; Lopes et al., 2000), non-volatile and not detected in the GC–MS analysis.

Finally, other compounds, such as α-tocopherol, identified based on the characteristic fragmentations of the TMS derivative (Khallouki et al., 2003), glycerol, isoprene derivates and alkanes (mainly heptacosane and nonacosane), among others were also identified.

4. Conclusions

The present work constitutes, to our knowledge, one of the first complete studies of the lipophilic extractives composition of different morphological parts of banana plant "Dwarf Cavendish". The different morphological parts of this plant, and particularly, leaf blades, present a high content of lipophilic extractives.

The high content of lipophilic extractives in banana plant residues can be problematic, if this raw material is used for fibre production (Cordeiro et al., 2004) and particularly, if used for pulp production (Back and Allen, 2000). However, due to the high content of steryl glucosides (Oliveira et al., 2005) together with the abundance of other sterol type structures, unsaturated fatty acids and cinnamic type acids, this plant can be considered as a good source of a valuable phytochemicals to be used for example in functional foods, which would represent a major contribution for the valorisation of "Dwarf Cavendish" plant residues.

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