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Characterization of chemical composition of some bryophytes common in Latvia

Laura Kļaviņa[1]*, Oskars Bikovens[2], Iveta Šteinberga[1], Viktorija Maksimova[3], Linda Eglīte[1]

[1]Department of Environmental Sciences, University of Latvia, Raiņa Bulv. 19, Rīga LV–1586, Latvia
[2]Latvian State Institute of Wood Chemistry, Dzērbenes 27, Rīga LV–1006, Latvia
[3]Faculty of Chemistry, University of Latvia, Raiņa Bulv. 19, Rīga LV–1586, Latvia

*Corresponding author, E-mail: laura.klavina1@gmail.com

Abstract

Bryophytes are the second largest taxonomic group in the plant kingdom, but studies conducted to better understand their chemical composition are limited and scattered. The aim of this paper is to determine and compare the chemical composition of bryophytes common in Latvia using elemental analysis, Fourier-transform infra-red and analytical pyrolysis–gas chromatography/mass spectrometry. Cluster analysis was used to better understand differences in chemical composition of bryophyte samples. Chemical analysis, Fourier-transform infra-red and pyrolysis–gas chromatography/mass spectrometry coupled with cluster analysis can be used only as rough tools for moss chemical taxonomy. It is possible that the differences in the composition of the studied mosses were determined by presence of secondary metabolites and not so much by their basic structural elements. Significant differences between Sphagnum species and other bryophytes were found.

Key words: bryophytes, chemical composition, elemental composition, pyrolysis.
Abbreviations: HCA, hierarchical cluster analysis; Py-GC/MS, pyrolysis–gas chromatography/mass spectrometry; FT-IR, Fourier-transform infra-red.

Introduction

Bryophytes are the second largest group in the plant kingdom with about 15 000 to 25 000 bryophyte species. Bryophytes can be found in any habitat globally where photosynthesis is possible. They are divided in three classes – mosses, liverworts and hornworts. The study of bryophyte biology and ecology has been receiving growing attention worldwide and has major importance in the understanding not only of this group of plants, but also in identification of basic ecological relations and study of development processes of living organisms (Glime 2007; Goffinet, Shaw 2008). An important direction in bryophyte studies is analysis of their biologically active substances, particularly the secondary metabolites. Recent studies have found high amounts of terpenoids, phenolics, glycosides, lipids (Rauha et al. 2000) in bryophyte samples. Bryophyte extracts have demonstrated antimicrobial, antifungal, cytotoxic and many other kinds of biological activities (Basile et al. 1999; Asakawa 2007; Selvam et al. 2010) and the number of studies on biologically active compounds in bryophytes is rapidly growing, resulting in identification of a large number of specific substances with high biological activity (Mellegård et al. 2009; Üçüncü et al. 2010).

It can be hypothesized that the chemical composition and presence of biologically active ingredients in bryophytes determine their stability in respect to degradation and that they influence soil and peat development processes and properties. Despite the abundance of bryophytes, the number of studies on their chemical composition is low, especially regarding basic structural elements (Zinsmeister, Mues 1990), such as carbohydrates, lignin residues, lipids, proteins and others. Carbohydrates are considered to be among the major ingredients of bryophytes, whereas the content of lignins has been found to be small, despite controversies also in this respect (Erickson, Miksche 1974; Ballance et al. 2008). It has been suggested that some carbohydrates can be a major factor determining biological stability of bryophytes and their ability to bind amino acids in the structure of proteins causing their inactivation (Bland et al. 1968). However, other studies contradict this, and emphasize the role of carboxylic groups bearing carbohydrates uronic acid residues in the major structural units (Hájek et al. 2011).

The chemical composition of bryophytes differs depending on species, growth environment and season, and information on chemical composition may provide an additional dimension in the study of bryophyte biology and ecology (Bragazza, Freeman 2007). Of importance is also information on elemental composition of bryophytes, including the major structural elements (carbon, nitrogen, oxygen, sulphur, hydrogen) and trace elements. Trace

element composition of bryophytes is widely used to determine the levels of pollutants in the environment (Rühling, Tyler 1970; Bragazza, Freeman 2007; Dmuchowski, Bytnerowicz 2009; Schröder et al. 2010).

In this respect, comparison of background concentrations and differences in concentrations between different species can be valuable in the understanding of bryophyte composition and properties (Zechmeister 2004).

Despite the importance of bryophyte chemistry studies, the number of studies in which common methods such as pyrolysis-gas chromatography (Py-GC/MS) and Fourier-transform infra-red spectrometry (FT-IR) have been used in structural characterization of biological material is low and fragmentary (Kracht, Gleixner 2000).

The aim of this paper is to analyze and compare the chemical composition of some common in Latvia bryophytes using elemental, spectral and destructive analytical methods.

Materials and methods

Bryophyte sampling

Fresh bryophyte material from eleven bryophyte species was collected in the growing season of 2011 in Sudas bog (57.15°N, 25.05°E), Cenas bog (56.88°N, 23.84°E) and their surrounding coniferous and mixed forests, and in coniferous forest near Kabile (56.93°N, 22.28°E) (Latvia). A list of the studied species and their growth conditions are given in Table 1. Only living material with a bright green colour without signs of decomposition was collected. The approximate amount of collected bryophyte material was 40 g dry weight per species. The moss samples were stored at –20 °C. Before analysis the material was cleaned and dried to constant weight at room temperature (24 °C) for 24 h. Samples for infrared spectrum, elemental analysis and Py-GC/MS analysis were dried, ground with a grinder and sieved through a 0.25 mm sieve.

Chemical analysis of bryophyte samples

Elemental analysis (C, H, N, S and O) of moss samples was carried out in triplicate using an Elemental Analyzer Model EA-1108, and the determined values were normalized with respect to ash content. Ash content was measured after heating 50 mg of each sample at 750 °C for 8 h. Fourier transform infrared spectra were obtained using a Nicolet AVATAR 330 spectrophotometer in KBr pellets.

The Py-GC/MS analysis was performed using a micro-furnace Frontier Lab Micro Double-shot Pyrolyser (Py-2020iD). The final pyrolysis temperature was 500 °C, heating rate 600 °C s^{-1}. The pyrolyser was directly coupled with a Shimadzu GC/MS-QP 2010 apparatus fitted with a capillary column RTX-1701 (60 m × 0.25 mm) and a 0.25 μm stationary phase film. The injector temperature was set at 250 °C, ion source 250 °C with EI of 70 eV, and MS scan range m/z 15-350. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1} and a split ratio 1 : 30. The mass of the sample was 1 to 2 mg. The oven program was 1 min isothermal at 60 °C, then 6 °C min^{-1} to 270 °C, and finally 10 min at 270 °C. The identification of individual compounds was performed on the basis of a GC/MS chromatogram using Library MS NIST 147.LI13. The total molar areas of the relevant peaks were normalized to 100%, and the data from three repetitive pyrolysis experiments were averaged.

Dried bryophyte sample (0.5 g) was acid digested with 25 mL of 50 % HNO_3 and 5 mL H_2O_2 and heated in a steam bath until the mixture volume was halved. Then additional 25 mL of 50% HNO_3 were added and the sample was heated until boiling. Thereafter, the solution was cooled and filtered and the filtrate was diluted to 50 mL with distilled water (Herber, Stoepller 1994). Metal concentrations were measured by graphite furnace atomic absorption (PerkinElmer AAnalyst200). Quality control was conducted using reference material: CRM 482 lichen. Accuracy was between 1 to 10 % for major elements and 1 to 2 % for trace elements. Detection limits were lower than 1 mg kg^{-1} for major elements.

Table 1. List of studied bryophyte species with codes and their growth conditions

Species	Code	Growth conditions
Aulacomnium palustris (Hedw.) Schwagr.	AP	Bog and humid places with soil pH 3.7 to 7.9
Polytrichum commune Hedw.	PC	Coniferous forests, moss bogs, humid places with soil pH 3.3 to 5.6
Polytrichum juniperum Hedw.	PJ	Coniferous forests, moss and transitional bog, grey dunes with soil pH 4 to 5.9
Ptilium crista-castrensis (Hedw.) De Not.	PCC	Mixed-wood forest, grassland, soil pH 3.2 to 4.5
Pleurozium schreberi (Willd. ex Brid.) Mitt.	PS	Base of timber, decaying wood, poor soil, substrate pH 3.3 to 7.2
Rhytidiadelphus triquetrus (Hedw.) Warnst.	RT	Mixed-wood forest, calcareous grasslands, grey dunes, soil pH 3.4 to 7.1
Sphagnum girgensohnii Russow	SG	Humid coniferous forests, grows together with *Polytrichum commune*
Sphagnum magellanicum Brid.	SM	Humid coniferous forests, bog
Sphagnum capillifolium (Ehrh.) Hedw.	SS	Edge of bog / Bog border
Sphagnum angustifolium (C.E.O. Jensen ex Russow) C.E.O. Jensen	SZ	Edge of bog / Bog border
Plagiochila asplenioides (L. Emend. Taylor) Dumort	PA	Coniferous forests, mixed-wood forest, substrate acidic or alkaline soil, peat, decaying wood

Data analysis

Hierarchical cluster analysis (HCA) was used to identify relatively homogeneous clusters of samples based on their similarity in measured characteristics (Steinbach et al. 2003). The agglomerative procedure starts with each object in a separate cluster and then combines the clusters sequentially, reducing the number of clusters at each step until all objects belong to only one cluster (Downs, Barnard 2003). We used Ward's method (Ward 1963) of hierarchical clustering, also known as the minimum variance method, which uses an analysis of variance approach to evaluate the distances between clusters. In short, beginning with N clusters consisting exactly of one entity, the similarity matrix is searched for the most similar pair of clusters and the number of clusters is reduced by one by merging the most similar pair of clusters with the minimum increase in the total within group error sum of squares (Hervada-Sala, Jarauta-Bragulat 2004). The analysis was performed using IBM SPSS Statistics 19.

Results and discussion

The studied bryophytes showed relatively low variability in their elemental composition (Table 2). The ranges in concentrations of basic elements in the studied bryophytes were: C 40 to 43%; H 5.5 to 6%; N 0.4 to 2%; S ~0%. The O content ranged between 48 to 53%, (as determined by mass balance). For comparison, the elemental compositions of moss peat have been determined to be: C 45 to 63%; H 3.6 to 7.7%; N 0.4 to 5.8%; S 0.5 to 1.5% (Zaccone et al. 2007). Thus, there was high similarity in the basic organic structural molecules of the studied bryophytes. However, there were some specific differences, such as a low concentration of nitrogen in *Sphagnum* species, in comparison with the other bryophytes, what can be explained with different growth conditions or other physiological or environmental differences which are not clear yet.

An important group of elements characterizing composition of bryophytes are major and trace metallic elements (Table 3). The sources of major elements are mostly natural processes, while presence of many trace elements can be due to environmental pollution (Berg, Steinnes 1997). However, among the studied metals, those directly associated with pollution (Pb, Cd) have the lowest variability and low concentrations, but concentrations of major elements (Na, K, Ca, Mg, Fe, Mn) and also essential trace elements with predominant natural origin (Ni, Cu, Zn) have the highest variability among the bryophyte species. These results show that pollution that is connected with the trace elements is low in regions were the bryophytes were collected.

The FT-IR spectra of the analyzed bryophytes (Fig. 1) can be divided into regions depending on the presence of functional groups. Absorption bands in the 3600 to 2800 cm^{-1} spectral region were very broad; absorbance in this region was determined by the presence of –OH groups. Sorption at wavelengths 2950 and 2850 cm^{-1} identified the presence of CH_3– and CH_2–groups, respectively. Typical minor sorption lines were common for the region around 1735 to 1700 cm^{-1}, which is characteristic for carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents and hydrogen bonding. In the spectral region 1690 to 1500 cm^{-1} it was possible to identify the sorption maxima of amide bonds (1640 to 1620 cm^{-1} and 1550 to 1540 cm^{-1}). In the region 1625 to 1610 cm^{-1}, the sorption indicated the presence of aromatic C=C and carbonyl groups, and quinones. At the wavelengths 1470 to 1370 cm^{-1}, there were bands typical for C–H and O–H bonding and sorption maximums typical for C–O. For the wavelengths < 1000 cm^{-1} fingerprint patterns were evident. Sorption in this spectral region provides information about the possible proportion of carbohydrate. Sorption at 1080 cm^{-1} showed OH deformation or C–O stretch of phenol and alcohol OH groups, and 1040 cm^{-1} indicated C–O stretch of polysaccharide components.

FT-IR spectra of the studied bryophytes (see Fig. 1 for spectra of four species) demonstrate evident similarities

Table 2. Elemental composition (%) of studied bryophyte species. Data are means from three samples

Species	Code	C	H	N	O
Aulacomnium palustre	AP	43.51	5.72	0.51	50.25
Polytrichum commune	PC	43.79	6.06	2.02	48.13
Polytrichum juniperum	PJ	41.99	5.89	1.99	50.14
Ptilium crista-castrensis	PCC	42.25	5.68	1.21	50.87
Pleurozium schreberi	PS	43.15	5.52	1.12	50.21
Rhytidiadelphus triquetrus	RT	42.47	5.56	1.12	50.85
Sphagnum girgensohnii	SG	42.04	5.74	0.85	51.17
Sphagnum magellanicum	SM	42.21	5.55	0.52	51.72
Sphagnum capillifolium	SS	40.98	5.58	0.42	53.02
Sphagnum angustifolium	SZ	41.78	5.52	0.43	52.27
Plagiochila asplenioides	PA	41.97	5.63	0.92	51.48

Table 3. Major and trace elements (mg kg^{-1}) in studied bryophyte species. Data are means from 3 samples. Code of studied species as in Table 1

Code	Na	Mg	K	Ca	Fe	Mn	Ni	Cu	Zn	Cd	Pb
AP	178	1254	4641	2662	170	262	8.2	6.5	31	0.20	3.2
PC	87	944	6210	2561	779	126	1.7	8.1	42	0.17	3.0
PJ	405	1344	3140	3390	80	38	0.6	18.4	179	0.21	1.7
PCC	226	1287	10123	4126	103	256	1.8	14.1	48	0.26	1.1
PS	24	1023	4779	3182	157	282	0.3	4.2	34	0.33	1.4
RT	171	1693	7311	3963	64	241	0.9	5.5	87	0.23	1.9
SG	253	1128	7535	2912	83	171	1.4	3.5	190	0.22	2.4
SM	643	1040	2739	3695	507	28	1.3	5.0	42	0.10	5.5
SS	615	1697	3637	6341	162	50	1.0	12.4	88	0.11	6.6
SZ	572	1093	5073	2801	123	29	0.6	3.3	71	0.05	2.3
PA	341	2001	20104	5583	152	179	2.8	9.4	39	0.30	2.5

in major sorption lines, but differ in their intensity, as well as in intensity of minor sorption lines. Thus, FT-IR spectra also indicate similarity in the composition of the studied bryophytes. However, the obtained differences do suggest that spectroscopic analysis can be used as a tool to determine differences between species. It is likely that the differences in the composition of studied mosses are not determined so much by their basic structural elements, but rather by the presence of secondary metabolites.

Pyrolysis-gas chromatography/mass spectrometry (van Smeerdijk and Boon 1987; Schellekens et al. 2009) was used to characterize the chemical composition of the bryophytes. Appendix 1 lists the components in the studied bryophyte volatile pyrolysates. The presence of dominant product groups from each bryophyte species is illustrated in Table 4. The pyrolysates of the studied *Sphagnum* species were dominated by carbohydrate decomposition products and simple phenolic compounds. These Py/GC-MS data are in conformity with the results of elemental analysis and FTIR spectroscopy. Areas of other identified peaks were summed and normalized to 100% in order to characterize the chemical composition of bryophytes.

The dominant compounds were low molecular weight aliphatic compounds, phenol and 4-ethenylphenol and 1,6-anhydro-β-D-glucopyranose. Their origin is likely carbohydrates and polyphenolics. The importance of cellulose in *Sphagnum* structures is indicated by the high abundance of polysaccharide products. No lignin markers were detected. The importance of cellulose in *Sphagnum* structures is indicated by the high abundance

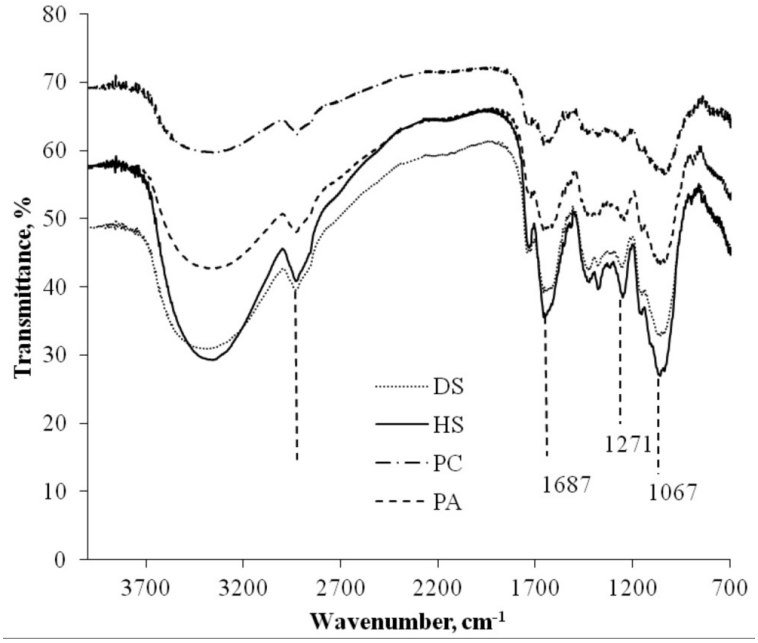

Fig. 1. FT-IR spectra of studied bryophytes *Dicranum scoparium*, DS; *Hylocomnium splendens*, HS; *Polytrichum commune*, PC; *Plagiochila asplenioides*, PA.

Table 4. Relative abundance (%) of the main groups of pyrolysis products of bryophytes. Code of studied species as in Table 1. MA, multi-origin aliphatic compounds with C<6; C, furan originated from carbohydrates, pyran and cyclopentene derivatives; Ar, aromatic compounds (except methoxylated phenols); L, methoxylated phenols; Lp, compounds originated from lipids C > 6; N, N-bearing compounds

Code	MA	C	Ar	L	Lp	N
AP	14.30	10.39	0.76	0	1.17	0
PC	17.03	6.05	0.72	0	1.97	0.08
PJ	16.16	5.75	0.60	0	1.23	0
PCC	19.90	4.74	0.79	0	1.45	0
PS	20.68	5.96	2.52	0	1.35	0
RT	16.68	5.83	3.21	0.18	0.96	0.05
SG	16.87	5.58	4.26	0	1.47	0.02
SM	14.26	9.12	3.48	0	1.24	0
SS	15.84	7.72	3.06	0	1.35	0
SZ	17.72	6.38	3.78	0	1.10	0
PA	14.53	3.36	1.16	1.02	3.98	0.11

of polysaccharide products, eg. methylglyoxal, 2,3 – butanedione, 2-hydroxyacetaldehyde, acetic acid and other low molecular weight pyrolysis products.

Other studied bryophyte species had pyrolysis products similar to the *Sphagnum* species, although with reduced phenol abundance and absence of 4-isoprenylphenol. Abundant polysaccharide products confirm the importance of cellulose in the structural make-up of bryophytes. No lignin markers were detected, thus indicating a major difference in comparison with composition of higher plants. As one of the main products, *Polytrichium* and *Bryopsida* species contained more acetic acid and methyl esters in comparison with *Sphagnum* species. Acetic acid resulted from the elimination of acetyl groups originally linked to the hemicelluloses xylose unit, the ring-scission of xylose and uronic acid residues (van Smeerdijk, Boon 1987). In distinction to other moss species, volatile pyrolysates of liverwort *Plagiochila asplenoides* contained cyclic aliphatic compounds indicating high content of terpene in liverwort.

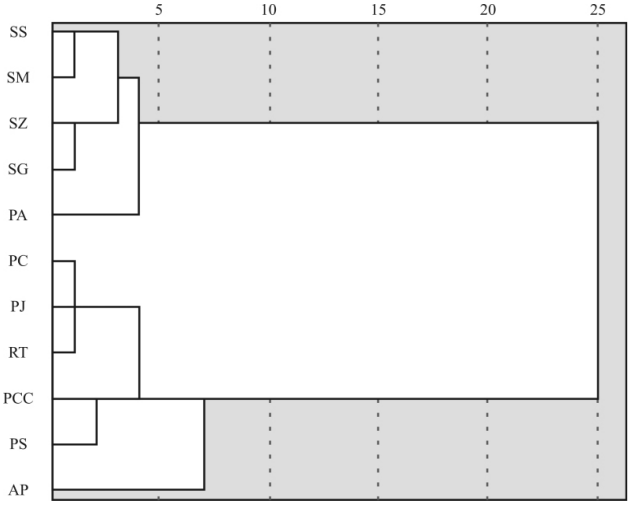

Fig. 2. Cluster analysis of amounts of pyrolysis products of bryophytes. Code of studied species as in Table 1.

Other studied pyrolysates were dominated by phenols, 2-methoxyphenols (guaiacol units), 2,6 dimethoxyphenols (syringyl units) and polysaccharide pyrolysis products. The high abundance of 4-ethenylphenol and 4-ethenyl-2-methoxyphenol indicated the presence of angiosperm ligno-cellulose.

Cluster analysis of pyrolysis products indicates similarity of major structural elements among groups of the studied bryophytes (Fig. 2). For example, despite the evident similarity in elemental composition, the *Sphagnum* species were grouped together. The only studied liverwort *Plagiochila asplenioides* was similar to the *Sphagnum* species, while the other studied bryophyte species formed another group. However, minor structural elements are also important in chemical taxonomy of bryophytes, particularly regarding substances belonging to lipid class (Appendix 1).

Today bryophytes have increasing importance as sources of valuable substances for biomedical applications. In the present study common bryophytes in Latvia were studied using different analytical methods. The main part of bryophyte biomass was composed of various carbohydrates. It can be concluded from the study that the general composition of mosses is very similar, as shown by their elemental composition and results of pyrolysis gas chromatography/mass spectrometry and FT-IR spectrometry, but differences in minor structural elements suggest that chemical analytical methods can be used to support taxonomy of mosses.

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Appendix 1. Peak assignments and relative abundance (%) of pyrolysis products of bryophytes. M+, Mass of molecular ion; MA, multi-origin aliphatic compounds with C < 6; C, carbohydrates originated furan, pyran and cyclopentene derivatives; Ar, aromatic compounds (except methoxylated phenols); L. methoxylated phenols; Lp, lipid originated compounds C > 6; N, N-bearing compounds

Peak No	Compound	M+	Origin	AP	PC	PJ	PCC	PS	RT	SG	SM	SS	SZ	PA
1	Carbon dioxide	44	–	27.62	28.20	29.97	29.53	27.45	29.09	30.31	29.77	29.27	29.98	31.24
2	Water	18	–	42.65	43.41	43.32	42.19	40.30	41.98	39.38	39.43	40.02	38.80	41.51
3	Furan	68	C	0.07	0.04	0.03	trace	0.15	0.02	0.01	0.01	0.05	0.04	0.02
4	Methylglyoxal	72	MA	1.80	2.12	1.92	2.00	2.21	2.16	2.71	3.09	3.46	3.26	2.25
5	2-Methylpropanal	72	MA	0.05	0.13	0.10	0.07	0.05	0.12	0.07	0.03	0.03	0.06	0.11
6	2-Methylfuran	82	C	0.46	0.29	0.29	0.27	0.31	0.30	0.18	0.24	0.21	0.19	0.13
7	2,3-Butanedione	86	MA	0.67	0.91	0.90	0.98	0.90	0.87	1.00	0.89	0.99	1.01	1.02
8	1-Hydroxy-2-butanone	88	MA	0.09	0.05	0.03	0.06	0.08	0.09	0.09	0.14	0.16	0.18	0.07
9	Benzene	78	Ar	0.07	0.05	0.05	0.05	0.06	–	–	trace	trace	–	–
10	2 or 3-Methylbutanal	86	MA	0.07	0.12	0.08	0.07	0.06	0.08	0.04	0.02	0.02	0.02	0.03
11	2-Hydroxyacetaldehyde	60	MA	0.34	0.48	0.46	0.48	0.56	0.55	1.33	1.51	1.61	1.47	0.60
12	2,5-Dimethylfuran	96	C	0.07	0.06	0.06	0.03	0.01	0.07	–	–	0.04	0.01	0.03
13	3-Methylbut-3-en-2-one	84	MA	0.06	0.06	0.03	0.05	0.06	0.06	0.02	0.08	0.03	0.03	0.02
14	Acetic acid	60	MA	8.76	8.11	8.67	10.67	11.66	8.20	5.91	3.54	4.02	5.05	5.11
15	2,3-Pentanedione	100	MA	0.05	0.06	0.08	0.08	0.07	0.06	0.13	0.12	0.15	0.13	0.10
16	1-Hydroxypropan-2-one	74	MA	1.03	2.39	1.99	2.30	2.32	1.97	2.47	1.83	2.48	3.05	3.51
17	Methylbenzene	92	Ar	0.14	0.09	0.04	0.03	0.06	0.11	0.04	0.01	trace	trace	0.06
18	Methyl formate	60	MA	–	–	–	–	–	0.11	trace	0.08	0.05	0.05	–
19	3-Hydroxybutan-2-one	88	MA	–	–	–	–	–	0.16	0.05	0.09	0.13	0.12	0.06
20	Propanoic acid	74	MA	0.09	0.16	0.15	0.18	0.26	0.07	0.14	0.14	0.13	0.15	0.23
21	Cyclopentanone	84	C	0.03	0.03	0.04	0.04	0.04	0.04	0.05	0.03	0.04	0.04	0.05
22	Methyl acetate	74	MA	0.25	0.48	0.37	0.53	0.50	0.53	0.56	0.75	0.70	0.70	0.31
23	Pyrrole	67	N	trace	0.08	trace	trace	trace	0.05	0.02	trace	trace	trace	0.11
24	1,4-Dimethylbenzene	106	Ar	0.05	0.02	0.05	0.04	0.07	trace	trace	trace	trace	trace	0.04
25	2(3H)-Furanone	84	C	0.04	0.06	0.06	0.07	0.06	0.05	0.07	0.07	0.05	0.06	trace
26	3(2H)-Furanone	84	C	0.31	0.13	0.32	0.12	0.12	0.28	0.29	0.52	0.25	0.19	0.12
27	1,2-Dimethylbenzene	106	Ar	0.05	trace	0.03	0.03	0.04	–	–	–	–	–	–
28	Methyl-2-oxopropanoate	102	MA	0.75	1.64	1.11	1.42	1.57	1.37	2.11	1.72	1.62	2.17	0.77
29	Furfural	96	C	1.15	0.72	1.14	0.85	1.07	0.70	0.91	1.25	0.84	0.91	0.66
30	2-Methyloctan-1-ol	144	Lp	0.10	0.07	0.05	0.17	0.09	0.15	trace	0.08	0.07	0.05	0.11
31	3-Methylbutanoic acid	102	MA	0.04	0.08	0.06	0.06	0.07	0.08	0.07	0.05	0.02	0.06	0.07
32	2-Furanmethanol	98	C	0.05	0.37	0.08	0.07	0.08	0.07	0.06	0.03	0.03	0.05	0.06
33	2-Oxopropyl acetate	116	MA	0.16	0.20	0.18	0.21	0.25	0.16	0.14	0.14	0.21	0.17	0.23
34	2-Methylcyclopent-2-en-1-one	96	C	0.05	0.06	0.08	0.07	0.07	0.06	0.05	0.04	0.05	0.06	0.08
35	3-Methylheptan-2-one	128	Lp	0.02	0.06	0.04	0.05	0.04	0.04	0.05	0.04	0.04	0.05	0.02
36	Acetylfuran	110	C	0.03	0.07	0.07	0.07	0.05	0.06	0.06	0.04	0.05	0.06	0.12
37	Limonene	136	Lp	0.03	0.05	trace	0.04	0.30	0.05	trace	0.04	trace	trace	trace
38	1,2-Cyclopentanedione	98	C	0.31	0.80	0.45	0.54	0.61	0.53	0.77	0.61	0.60	0.67	0.41
39	5-Methylfuran-2-carbaldehyde	110	C	0.15	0.15	0.20	0.08	0.07	0.07	0.17	0.21	0.10	0.09	trace
40	2-Oxobutyl acetate	130	MA	0.05	0.04	0.03	0.03	0.06	0.04	0.03	0.04	0.03	0.04	0.04
41	3-Methylcyclopent-2-en-1-one	96	C	0.09	0.10	0.12	0.10	0.10	0.11	0.10	0.08	0.10	0.11	0.11
42	Dihydro-2(3H)-furanone	86	C	trace	0.11	trace	0.08	0.09	0.10	0.07	0.07	0.07	0.07	0.06
43	2H-furan-5-one	84	C	0.06	0.29	0.13	0.22	0.20	0.19	0.17	0.18	0.19	0.19	0.07
44	3-Hydroxy-5,6-dihydro-(4H)-pyran-4-one	114	C	0.34	0.07	0.15	0.13	0.17	0.13	0.30	0.95	0.52	0.31	0.07
45	Octen-1-ol acetate	170	Lp	–	–	–	–	–	–	–	–	–	–	0.12
46	2,3-Dimethylcyclopent-2-en-1-one	110	Lp	0.04	0.05	0.06	0.08	0.07	0.07	0.04	0.04	0.05	0.06	0.09
47	3-Methylcyclopentane-1,2-dione	112	C	0.12	0.44	0.50	0.52	0.48	0.52	0.45	0.31	0.34	0.43	0.45
48	Nonanal	142	Lp	0.06	trace	trace	–	–	–	–	–	–	–	–
49	3,5-dimethyloctane	142	Lp	–	–	–	0.05	0.03	–	–	–	–	–	–
50	Phenol	94	Ar	0.17	0.21	0.15	0.17	1.17	1.66	2.55	1.84	1.75	2.22	0.25

Appendix 1. *continued*

Peak No	Compound	M+	Origin	AP	PC	PJ	PCC	PS	RT	SG	SM	SS	SZ	PA
51	2-Methoxyphenol (guaiacol)	124	L	trace	trace	trace	trace	trace	0.11	trace	trace	–	–	0.25
52	4-Hydroxy-2,5-dimethylfuran-3-one	128	C	0.22	0.16	0.19	0.13	0.13	0.11	0.16	0.26	0.20	0.13	trace
53	2-Methylphenol	108	Ar	0.07	0.09	0.08	0.06	0.17	0.17	0.18	0.19	0.16	0.22	0.09
54	3-Ethyl-2-hydroxy-cyclopent-2-en-1-one	126	Lp	0.07	0.04	0.06	0.06	0.04	0.04	0.06	0.02	0.05	0.07	0.10
55	3-Hydroxy-2-methylpyran-4-one	126	C	trace	0.05	trace	0.11	0.05	0.13	0.12	0.13	0.14	0.10	trace
56	Undecace	156	Lp	0.04	–	trace	0.04	0.04	–	–	–	–	–	–
57	3 or 4-methylphenol	108	Ar	0.14	0.17	0.15	0.18	0.31	0.50	0.37	0.40	0.39	0.30	0.44
58	p-Methylguaiacol	138	L	trace	trace	trace	trace	–	0.07	–	trace	trace	–	0.06
59	3,4-Dimethylphenol	122	Ar	–	–	–	–	–	0.04	0.04	0.04	0.03	0.05	trace
60	Pentanal	86	C	0.32	0.92	0.46	0.56	0.74	0.66	0.72	0.43	0.40	0.70	0.52
61	3,7-Dimethylnonane	156	Lp	0.04	–	0.02	0.05	0.04	–	–	–	–	–	–
62	4-Ethylphenol	122	Ar	0.04	0.05	0.05	0.06	0.10	0.13	0.17	0.20	0.18	0.17	trace
63	2-Propylheptan-1-ol	158	Lp	0.45	0.19	0.23	0.35	0.24	0.34	0.34	0.69	0.93	0.48	-
64	Guaia-1(10),11-diene	204	Lp	–	–	–	–	–	–	–	–	–	–	0.31
65	1,4:3,6-Dianhydro-	144	C	0.22	0.20	0.15	0.18	0.16	0.21	0.13	0.23	0.16	0.16	trace
66	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-3-ene	204	Lp	–	–	–	–	–	–	–	–	–	–	0.18
67	Selina-3,7(11)-diene	204	Lp	–	–	–	–	–	–	–	–	–	–	0.06
68	Guaia-1(10),11-diene (isomer)	204	Lp	–	–	–	–	–	–	–	–	–	–	0.44
69	p-Vinylguaiacol	150	L	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	0.71
70	2,3-Dihydro-1-benzofuran	120	Ar	0.03	0.04	–	0.02	0.02	0.21	0.42	0.35	0.29	0.34	trace
71	Pentadecane	212	Lp	0.11	0.05	0.05	0.12	0.16	0.03	trace	0.04	0.03	0.05	–
72	Humulen	204	Lp	–	–	–	–	–	–	–	–	–	–	0.05
73	Humulen (isomer)	204	Lp	–	–	–	–	–	–	–	–	–	–	0.34
74	5-(Hydroxymethyl) furan-2-carbaldehyde	126	C	0.25	trace	0.04	0.04	0.24	0.04	trace	0.37	0.28	0.47	–
75	2-Methyl-2,3-dihydro-1-benzofuran	134	Ar	–	–	–	–	–	–	0.49	0.45	0.26	0.48	–
76	p-Methylsyringol	168	L	–	–	–	–	–	–	–	–	–	–	trace
77	Epiglobulol	222	Lp	–	–	–	–	–	–	–	–	–	–	0.07
78	Spathulenol	220	Lp	–	–	–	–	–	–	–	–	–	–	0.15
79	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-	170	Lp	–	–	–	–	–	–	–	–	–	–	0.83
80	3-Hexadecene	224	Lp	0.06	–	–	0.09	–	–	0.06	0.06	–	0.06	0.08
81	[1,1'-Biphenyl]-4-carboxaldehyde	182	Ar	–	–	–	0.15	–	–	–	–	–	–	0.28
82	3,7,11,15-Tetramethylhexadec-2-ene	280	Lp	–	0.13	0.08	0.07	–	–	0.07	0.03	–	0.04	0.10
83	1-(3-Hydroxyphenyl) ethanone	136	Ar	–	–	–	–	0.52	0.39	–	–	–	–	–
84	3,7,11,15-Tetramethylhexadec-2-en-1-ol	296	Lp	0.15	1.33	0.64	0.28	0.14	0.24	0.85	0.20	0.18	0.24	0.93
85	1,6-Anhydro-β -D-glucopyranose	162	C	6.05	0.93	1.19	0.46	0.96	1.38	0.74	3.06	3.01	1.34	0.40
86	2-Nonadecanone	282	Lp	–	–	–	0.10	–	–	–	–	–	–	–
87	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	410	Lp	0.58	0.70	0.83	0.29	0.58	0.63	0.20	0.28	0.53	0.41	0.44