JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2009,** *57,* 6795–6802 **6795**
DOI:10.1021/jf901532m

Volatile Constituents throughout *Brassica oleracea* L. Var. *acephala* Germination

Fátima Fernandes,[†] Paula Guedes de Pinho,*[,†] Patrícia Valentão,[†] José A. Pereira,[‡] and Paula B. Andrade*[,†]

[†]REQUIMTE/Department of Pharmacognosy, Faculty of Pharmacy, Porto University, R. Aníbal Cunha, 164, 4050-047 Porto, Portugal, and [‡]CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

In this work, the volatile composition of kale (*Brassica oleracea* L. var. *acephala*) and its variation during germination were monitored during the first 9 days of seedling development by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography/ion trap−mass spectrometry (GC/IT-MS). Differences were found among the materials in the distinct analyzed periods. A total of 66 volatile compounds, distributed in several chemical classes, were determined: alcohols, carbonyl compounds (ketones, aldehydes, and esters), norisoprenoids, and terpenes, among others, sulfur compounds being the most abundant group in seeds and sprouts that exhibited allyl isothiocyanate as the major compound. Leaves of fully developed ground plant had the highest content of norisoprenoids, alcohols, and carbonyl compounds; in opposition, they showed lower levels of sulfur compounds, suggesting that these are important molecules for the development of kale, whereas the others are produced mainly during its growth.

KEYWORDS: *Brassica oleracea* L. var. *acephala*; kale; leaves; sprouts; seeds; volatile compounds; HS-SPME; GC/IT-MS

INTRODUCTION

Leaves of the Brassicaceae family are commonly grown and consumed worldwide. Like leaves, seeds are used for human consumption as oil (canola seeds) or added to some food products (e.g., bread and cake). Sprouts, the germinating form of seeds, are nowadays also used as food and are favored for their nutritional value, becoming a familiar component in salads (*1*). Scientific attention on seeds and sprouts of these vegetables has increased because of their different kinds of bioactive constituents, which may act as dietary contributors for good health status (*2*). Among *Brassica* vegetables, kale (*Brassica oleracea* L. var. *acephala*) is important in traditional farming systems in the Iberian peninsula, and its leaves are consumed fresh or after cooking, as soup.

The benefits of *Brassica* vegetables' consumption arises from their high concentration of vitamins, minerals, and a special group of phytochemicals, glucosinolates, which co-occur with myrosinase isoenzymes and are associated with cancer protection (*2*, *3*). *Brassica* species have also been extensively studied for their typical flavor and odor, attributed to volatile sulfur compounds (*4*−*6*).

Glucosinolates constitute a main group of sulfur-containing plant secondary metabolites, which are relatively unique to cruciferous vegetables. When they come into contact with myrosinases, in the presence of water (during processing, cutting, tissue chewing, or when injured), glucosinolates are transformed into biologically active products (isothiocyanates, thiocyanates, nitriles, epithionitriles, and oxazolidines). Some of these hydrolysis products have a chemoprotective effect against certain cancers (*3*, *7*−*10*); however, they are also involved in goitrogenicity, although only in situations of iodine deficiency (*11*). Isothiocyanates produce a pungent flavor and sulfurous aroma, playing a significant organoleptic role in *Brassica* products (*12*, *13*). These compounds have been frequently mentioned, due to their health-promoting properties, as chemoprotective (*8*, *11*).

Besides breakdown glucosinolate products, other volatile metabolites (often monoterpenes, sesquiterpenes, and other aromatic compounds) are released from the surface of the leaf and/or from accumulated storage sites in the leaf. In addition, the green-leaf odor is attributed to a blend of saturated and unsaturated six-carbon alcohols, aldehydes, and esters that are released when leaves are mechanically damaged (*14*).

Several sulfides, polysulfides, thiols, nitriles, alcohols, carbonyl compounds, furans, and terpene hydrocarbons have been reported in *Brassica* vegetables (*15*), including in the seeds (*16*, *17*).

As far as we are aware, no previous work concerned the volatile composition of several stages of development of *B. oleracea* var. *acephala* (seeds, sprouts, and fully developed ground plant), and much of the existent literature focuses only on fully grown plants of other *B. oleraceae* crops (*4*−*6*, *15*, *17*).

As both sprouts and fully developed plants are used in the human diet, in this study we determined the volatile profile of seeds, seedlings of up to 6 and 9 days of age, and mature plants of *B. oleracea* var. *acephala*. Headspace solid-phase microextraction (HS-SPME), combined with gas chromatography/ion trap-mass

*Authors to whom correspondence should be addressed [telephone + 351 222078935; fax + 351 222003977; e-mail (P.B.A.) pandrade@ff.up.pt of (P.G.d.P.) pguedes@ff.up.pt].

Published on Web 07/17/2009

spectrometry (GC/IT-MS) was the analytical tool of choice, due to its feasibility regarding minimal sample size and preparation, fast sample throughput, and very high sensitivity for the simultaneous extraction of a broad range of analytes (*18*).

MATERIALS AND METHODS

Standards. Reference compounds were purchased from various suppliers: pentanal, hexanal, (*E*)-2-hexenal, octanal, (*E*)-2-octenal, ethyl octanoate, ethyl decanoate, ethyl linoleate, ethyl hexadecanoate, *trans*-geranylacetone, 6-methyl-5-hepten-2-one, 2,2,6-trimethylcyclohexanone, (*E*,*E*)-3,5-octadien-2-one, benzenepropanenitrile, β-cyclocitral, β-homocyclocitral, limonene, safranal, and eugenol were from Sigma-Aldrich (St. Louis, MO); (*E*)-2-decenal, (*E*,*Z*)-2,6-nonadienal, phenylacetaldehyde, β-ionone, α-ionone, dimethyl disulfide, dimethyl trisulfide, *n*-butyl isothiocyanate, hexyl isothiocyanate, 3-methylthiopropyl isothiocyanate, and phenylethyl isothiocyanate were obtained from SAFC (Steinheim, Germany); α-pinene, β-pinene, eucalyptol, and *o*-cymene were from Extrasynthese (Genay, France); menthol was obtained from Fluka (Buchs, Switzerland); and allylisothiocyanate was from Riedel de Haën (Seelze, Germany).

Samples. *B. oleracea* L. var. *acephala* seeds and fully developed plants were obtained in 2008 in Bragança, northeastern Portugal. Two hundred seeds were placed in 15 cm diameter Petri dishes lined with fiberglass and watered with 200 mL of distilled water to maintain approximately 100% relative humidity throughout the germination period. The seeds were germinated at 20−23.5 °C, under a 16 h light/8 h dark regimen. At 6 and 9 days of germination four plates were withdrawn.

Both sprouts and leaves were frozen (−20 °C) and freeze-dried (Labconco 4.5 Freezone apparatus, Kansas City, MO). All of the samples were powdered and stored in a desiccator in the dark.

SPME Fibers. Several commercial fibers can be used to extract volatiles. According to the bibliography, recommendations of the supplier (Supelco, Bellefonte, PA), and our knowledge (*19*, *20*), three of them are the most indicated for the intended compounds. The fibers used were coated with different stationary phases and various film thicknesses: carboxen/polydimethylsiloxane (CAR/PDMS), 75 μm; carbowax/divinylbenzene (CW/DVB), 65 μm; divinylbenzene/PDMS (DVB/PDMS), 65 μm. They were conditioned by inserting them into the GC injector; temperature and time were used according to Supelco's recommendation procedure: 300 °C for 1 h, 220 °C for 30 min, and 250 °C for 30 min, respectively.

HS-SPME. Approximately 0.1 g of each powdered sample was placed in a 15 mL vial, and 5 mL of 5% ethanol was added. The same procedure was tried with the same volume of water or without any solvent. The vial was then sealed with a polypropylene hole cap and PTFE/silicone septum (Supelco). The DVB/PDMS fiber was exposed to the headspace, and samples were stirred (150 rpm) at 45 °C for 20 min. Afterward, the fiber was pulled into the needle sheath, and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 1 min, the fiber was removed and conditioned in another GC injection port for 20 min, at 250 °C. The same procedure was used to test CAR/PDMS and CW/DVB fibers.

Gas Chromatography−Mass Spectrometry Analyses. HS-SPME analyses were performed using a Varian CP-3800 gas chromatograph equipped with a Varian Saturn 4000 mass selective detector and Saturn GC-MS workstation software version 6.8. A VF-5 ms (30 m × 0.25 mm × 0.25 μm) column from Varian was used. To check the identity of some of the compounds found with this column, a Stabilwax-DA fused silica (60 m × 0.25 mm, 0.25 μm) column (Restek) was also used. The injector port was heated to 220 °C. The injections were performed in splitless mode. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 mL/min. The oven temperature was set at 40 °C for 1 min, then increased at 2 °C/min to 220 °C, and held for 30 min. All mass spectra were acquired in electron impact (EI) mode. Ionization was maintained off during the first minute. The ion trap detector was set as follows: the transfer line, manifold, and trap temperatures were 280, 50, and 180 °C, respectively. The mass ranged from *m*/*z* 40 to 350, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25000 μs, with an ionization storage level of *m*/*z* 35. Analyses were performed in full-scan mode.

Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic standards analyzed under the same conditions and by comparison of the retention indices (as Kovats indices) with literature data (*4*, *15*, *17*). MS fragmentation patterns were compared with those of pure compounds, and mass spectrum database search was performed using the National Institute of Standards and Technology (NIST) MS 05 spectral database. Confirmation was also conducted using a laboratory-built MS spectral database, collected from chromatographic runs of pure compounds performed with the same equipment and conditions. For quantification purposes, each sample was injected in triplicate, and the results are expressed in areas (as Kcount amounts). Chromatographic peak areas were determined by a reconstructed full-scan chromatogram using for each compound some specific quantification ions (see **Table 1**): these corresponded to base ion (*m*/*z* 100% intensity), molecular ion (M^+), and another characteristic ion for each molecule. Some peaks that are coeluted in full-scan mode (resolution value < 1) can be integrated with a value of resolution > 1.

Statistical Analyses. Principal component analysis (PCA) was carried out using XLSTAT 2007.5 software. The PCA method shows similarities between samples projected on a plane and makes it possible to identify which variables determine these similarities and in what way.

RESULTS AND DISCUSSION

Analytical Conditions. Several studies recommend the application of HS-SPME for volatile profiling purposes in *Brassica* species (*15*, *18*) and other fields of plant science (*21*). HS-SPME analyses were performed using the odoriferous freeze-dried kale materials, once the high number of samples renders impractical the study of fresh samples in due time, because of their obvious alteration. In addition, samples were freeze-dried in the dark, which reduced the possibility of compound modification. The DVB/PDMS fiber was chosen as it was revealed to be the best and more selective one for the identification of sulfur compounds, glucosinolate breakdown products important in *Brassica* characterization (*7*).

It was previously demonstrated by our group that the better liquid/gas equilibrium for the majority of volatile compounds is obtained with 5% ethanol (*19*). Therefore, HS-SPME analyses were performed in the powdered materials mixed with this solution. In this way, less water-soluble compounds can also be released to the gas phase and a large range of compounds, with distinct polarities, is determined. We have also tried to analyze the headspace of the mixture with water only and the headspace of the dried material. However, with these last procedures lower amounts of compounds were detected; by superimposing the chromatograms it was possible to conclude that the use of 5% ethanol gave a most complete volatile profile. The presence of ethanol in the samples was excluded by GC/FID analyses; once,

Table 1. Volatile Compounds in Kale Seeds, Sprouts, and Fully Developed Plant

				A/1000 ± SD[d]			
compound	RI[a]	ID[b]	QI[c] (*m*/*z*)	seeds	6 days	9 days	leaves
alcohols							
1							
1-penten-3-ol	798	T	57	nd	nd	nd	7.0 ± 0.5
2							
(*E*)-2-nonenol	1216	T	57/70/96	1.7 ± 0.1	16.4 ± 1.3	26.6 ± 0.4	86.0 ± 4.0
aldehydes							
3							
pentanal	782	S	44/57/58	nd	12.7 ± 0.8	18.6 ± 1.1	14.3 ± 1.2
4							
(*E*)-2-pentenal	871	T	55/83	nd	nd	nd	9.4 ± 0.8
5							
hexanal	914	S	56/67/83	2.3 ± 0.1	5.5 ± 0.3	15.2 ± 0.7	32.9 ± 2.8
6							
(*E*)-2-hexenal	969	S	55/69/83	nd	nd	4.5 ± 0.3	152.8 ± 13.7
7							
heptanal	1014	T	55/70	nd	1.1 ± 0.1	2.8 ± 0.1	5.4 ± 0.3
8							
(*Z*)-4-heptenal	1071	T	57/70/83	nd	nd	2.7 ± 0.2	6.3 ± 0.0
9							
(*E*,*E*)-2,4-heptadienal	1109	T	53/81	nd	nd	nd	120.1 ± 7.3
10							
octanal	1116	S	67/81/95	nd	nd	3.4 ± 0.1	10.0 ± 0.9
11							
phenylacetaldehyde	1159	S	91	nd	36.8 ± 1.8	58.5 ± 2.3	40.0 ± 0.5
12							
(*E*)-2-cctenal	1172	S	70/93	nd	nd	nd	8.0 ± 0.7
13							
(*E*,*Z*)-2,6-nonadienal	1267	S	41/67/70	nd	nd	nd	4.4 ± 0.3
14							
(*E*)-2-decenal	1310	S	81/95	1.0 ± 0.0	5.8 ± 0.4	12.4 ± 0.9	11.1 ± 0.5
esters							
15							
butyl acetate	925	T	44/56/61	3.6 ± 0.1	6.7 ± 0.4	6.5 ± 0.1	13.7 ± 1.1
16							
ethyl benzoate	1284	T	77/105/122	nd	nd	19.0 ± 0.9	nd
17							
ethyl octanoate	1304		88/140	nd	nd	nd	15.3 ± 0.1
18							
ethyl decanoate	1405	S	88/157	nd	nd	nd	4.6 ± 0.0
19							
ethyl hexadecanoate	1907	S	88/157/284	nd	0.5 ± 0.0	1.6 ± 0.1	19.1 ± 1.8
20							
ethyl linoleate	1979	S	79	nd	nd	nd	23.8 ± 2.2
ketones							
21							
4-methyl-2-heptanone	1049	T	58/85	nd	2.4 ± 0.2	1.9 ± 0.1	nd
22							
3-octen-2-one	1152	S	55/97/111	nd	nd	nd	4.7 ± 0.2
23							
(*E*,*E*)-3,5-octadien-2-one	1205	S	71/105	nd	nd	nd	89.3 ± 8.1
norisoprenoid derivatives							
24							
6-methyl-5-hepten-2-one	1096	S	67/108	nd	nd	nd	12.6 ± 0.5
25							
2,2,6-trimethylcyclohexanone	1150	S	82/140	nd	nd	nd	12.0 ± 0.8
26							
isophorone	1174	T	82/110	nd	nd	nd	5.0 ± 0.4
27							
6-methyl-5-hepten-2-ol	1225	T	77/95/110	nd	nd	nd	66.6 ± 3.7
28							
safranal	1308	S	91/105	nd	4.8 ± 0.2	3.5 ± 0.2	3.6 ± 0.3
29							
β-cyclocitral	1320	S	109/137/152	nd	5.9 ± 0.2	10.6 ± 0.7	120.2 ± 11.4
30							
β-homocyclocitral	1340	S	107/151	nd	nd	nd	13.9 ± 0.8
31							

Table 1. Continued

compound	RI[a]	ID[b]	QI[c] (*m*/*z*)	*A*/1000 ± SD[d] seeds	6 days	9 days	leaves
α-ionone	1438	S	93/121	nd	nd	nd	3.3 ± 0.2
32							
trans-geranylacetone	1461	S	107	nd	nd	nd	12.4 ± 0.8
33							
β-ionone	1494	S	177	nd	6.6 ± 0.4	17.4 ± 0.7	331.7 ± 17.2
34							
5,6-epoxy-β-ionone	1497	T	177/123	nd	nd	nd	31.2 ± 1.2
35							
dihydroactinidiolide	1542	T	111/137/180	nd	nd	nd	20.0 ± 0.3
terpenic compounds							
36							
α-pinene	1047	S	53/93	1.9 ± 0.1	nd	nd	2.3 ± 0.1
37							
β-pinene	1099	S	69/93	nd	nd	nd	8.8 ± 0.8
38							
m-cymene	1126	T	91/119	nd	nd	nd	2.6 ± 0.1
39							
limonene	1143	S	67/93	0.5 ± 0.0	nd	6.8 ± 0.5	18.0 ± 0.9
40							
eucalyptol	1147	S	81/93/139	nd	nd	nd	9.2 ± 0.8
41							
o-cymene	1190	S	91/119	nd	nd	nd	3.3 ± 0.2
42							
menthol	1292	S	81/95/123	1.8 ± 0.1	9.8 ± 0.6	10.3 ± 0.5	5.2 ± 0.2
sulfur compounds							
43							
dimethyl disulfide	854	S	45/79/94	nd	4.8 ± 0.4	6.3 ± 0.3	nd
44							
isopropyl isothiocyanate	945	T	43/86/101	11.9 ± 0.5	10.5 ± 0.7	9.0 ± 0.5	nd
45							
allyl thiocyanate	975	T	41/72/99	82.6 ± 2.5	24.6 ± 1.6	29.8 ± 1.7	nd
46							
allyl isothiocyanate	997	S	41/72/99	64827.9 ± 2944.5	48642.1 ± 4131.6	53188.3 ± 2543.2	144.5 ± 7.7
47							
2-butyl isothiocyanate	1043	T	56/86	87.8 ± 2.8	112.4 ± 10.8	86.2 ± 5.0	nd
48							
isobutyl isothiocyanate	1066	T	57/72/115	937.3 ± 47.6	1083.5 ± 82.2	1052.3 ± 67.9	nd
49							
dimethyl trisulfide	1083	S	45/79/126	nd	16.6 ± 1.0	31.1 ± 1.6	nd
50							
3-butenyl isothiocyanate	1092	T	55/72/113	2907.0 ± 198.0	2042.1 ± 156.8	1901.2 ± 112.9	nd
51							
n-butyl isothiocyanate	1107	S	72/100/115	2.8 ± 0.2	4.2 ± 0.3	4.0 ± 0.1	nd
52							
3-methylbutyl isothiocyanate	1171	T	55/72/114	559.8 ± 37.1	802.0 ± 53.5	851.0 ± 36.0	nd
53							
pentyl isothiocyanate	1207	T	72/101/129	19.9 ± 1.5	34.3 ± 2.4	44.3 ± 1.6	nd
54							
4-methylpentyl isothiocyanate	1273	T	72/128/143	16.2 ± 0.6	30.8 ± 1.8	44.3 ± 2.3	nd
55							
hexyl isothiocyanate	1305	S	72/115/128	4.8 ± 0.3	18.5 ± 1.5	21.3 ± 1.2	nd
56							
3-methylthiopropyl isothiocyanate	1367	S	72/101/147	4010.5 ± 249.0	5108.6 ± 278.4	5661.7 ± 443.8	nd
57							
benzyl thiocyanate	1391	T	65/91	168.6 ± 12.8	305.1 ± 17.7	316.1 ± 16.3	nd
58							
phenylethyl isothiocyanate	1482	S	91/105/163	773.3 ± 52.1	1290.2 ± 67.2	1563.3 ± 40.9	1.4 ± 0.1
nitrogen compounds							
59							
3-methylisothiazole	1119	T	59/72/99	41.5 ± 2.3	196.2 ± 15.4	582.0 ± 9.7	nd
60							
4-(methylthio)butanenitrile	1195	T	61/115	25.1 ± 2.1	18.2 ± 1.2	75.4 ± 4.0	nd
61							
benzylnitrile	1254	T	51/90/117	nd	1.8 ± 0.1	5.3 ± 0.4	nd
62							
benzothiazole	1324	T	69/108/135	nd	nd	nd	8.7 ± 0.7

Table 1. Continued

compound	RI[a]	ID[b]	QI[c] (*m*/*z*)	*A*/1000 ± SD[d] seeds	6 days	9 days	leaves
63							
benzenepropanenitrile	1330	S	65/91/131	5.5 ± 0.3	37.0 ± 2.2	76.0 ± 2.2	nd
64							
2-methyl-3-(2*H*)-isothiazolone	1447	T	61/87/115	53.5 ± 2.1	35.9 ± 1.0	69.9 ± 2.1	nd
miscellaneous compounds							
65							
toluene	881	T	91/92	6.9 ± 0.4	17.6 ± 1.0	14.3 ± 0.9	26.4 ± 2.1
66							
eugenol	1386	S	164	nd	nd	nd	0.9 ± 0.1
identified compounds				26	35	40	45
alcohols (Σ)				1 (1.7)	1 (16.4)	1 (26.6)	2 (93.0)
aldehydes (Σ)				2 (3.3)	5 (61.9)	8 (118.0)	12 (414.8)
esters (Σ)				1 (3.6)	2 (7.2)	3 (27.1)	5 (76.6)
ketones (Σ)				0	1 (2.4)	1 (1.9)	2 (94.1)
norisoprenoid derivatives (Σ)				0	3 (17.3)	3 (31.5)	12 (632.5)
terpenic compounds (Σ)				3 (4.1)	1 (9.8)	2 (17.1)	7 (49.3)
sulfur compounds (Σ)				14 (74410.4)	16 (59530.4)	16 (64810.2)	2 (146.0)
nitrogen compounds (Σ)				4 (125.6)	5 (289.0)	5 (808.6)	1 (8.7)
miscellaneous compounds (Σ)				1 (6.9)	1 (17.6)	1 (14.3)	2 (27.3)

[a] Retention indices as determined on a HP-5 capillary column using the homologous series of *n*-alkanes. [b] Identification: T, tentatively identified by NIST05; S, identified by comparison with standard. [c] Quantification ions. [d] Mean area (in Kcounts) ± standard deviation of four replicates, analyzed in triplicate; nd, not detected.

when HS-SPME was applied to powdered samples and to an aqueous mixture, only vestigial amounts of ethanol were found (data not shown). Additionally, ionization was maintained off during the first minute of GC-MS analyses to avoid solvent overload. Ethanol eluted during this period, not influencing the elution of the samples' compounds.

Volatile Composition. The chromatographic profile of kale materials (**Table 1**) revealed the presence of 66 volatile compounds, distributed in several chemical classes: alcohols (**1**, **2**), carbonyl compounds (aldehydes (**3**−**14**), esters (**15**−**20**), and ketones (**21**−**23**)), norisoprenoids (**24**−**35**), terpenes (**36**−**42**), sulfur compounds (**43**−**58**), nitrogen compounds (**59**−**64**), and other volatiles (**65** and **66**). As far as we know, all of these compounds are described for the first time in kale. From these, 26 compounds were found in seeds and 35 and 40 in sprouts at 6 and 9 days, respectively (**Table 1**). Kale leaves exhibited the highest diversity of volatiles, presenting 45 compounds (**Table 1**). Compounds such as 1-penten-3-ol (**1**), (*E*)-2-pentenal (**4**), (*E*,*E*)-2,4-heptadienal (**9**), (*E*)-2-octenal (**12**), (*E*,*Z*)-2,6-nonadienal (**13**), ethyl octanoate (**17**), ethyl decanoate (**18**), ethyl linoleate (**20**), 3-octen-2-one (**22**), (*E*,*E*)-3,5-octadien-2-one (**23**), 6-methyl-5-hepten-2-one (**24**), 2,2,6-trimethylcyclohexanone (**25**), isophorone (**26**), 6-methyl-5-hepten-2-ol (**27**), β-homocyclocitral (**30**), α-ionone (**31**), *trans*-geranylacetone (**32**), β-ionone (**33**), epoxy-β-ionone (**34**), dihydroactinidiolide (**35**), *m*-cymene (**38**), eucalyptol (**40**), *o*-cymene (**41**), benzothiazole (**62**), and eugenol (**66**) were detected only in this matrix (**Table 1**). Additional differences were found between materials in the distinct development stages.

Seeds and sprouts mainly contained sulfur compounds. With the exceptions of dimethyl disulfide (**43**) and dimethyl trisulfide (**49**), detected only in sprouts, all sulfur compounds were present in both materials. Plants that produce glucosinolates commonly accumulate them in all vegetative and reproductive parts (*22*). Seeds exhibited the highest contents, followed by sprouts. Assuming glucosinolates function in defense against herbivores and pathogens, the differences among the several stages of development are consistent with current theories on optimal distribution of defense substances (*22*−*24*). The reproductive organs, including seeds and their germinating form (sprouts), which contribute most to plant fitness, are expected to have the highest concentrations of defense compounds (*22*).

Hexanal (**5**), (*E*)-2-hexenal (**6**), heptanal (**7**), (*E*,*E*)-2,4-heptadienal (**9**), octanal (**10**), phenylacetaldehyde (**11**), (*E*,*Z*)-2,6-nonadienal (**13**), (*E*,*E*)-3,5-octadien-2-one (**23**), 2,2,6-trimethylcyclohexanone (**25**), safranal (**28**), β-cyclocitral (**29**), β-homocyclocitral (**30**), α-pinene (**36**), β-pinene (**37**), limonene (**39**), allyl isothiocyanate (**46**), isobutyl isothiocyanate (**48**), dimethyl trisulfide (**49**), 3-butenyl isothiocyanate (**50**), 3-methylthiopropyl isothiocyanate (**56**), benzyl thiocyanate (**57**), phenylethyl isothiocyanate, (**58**), benzothiazole (**62**), and eugenol (**66**) were previously reported in the leaves of other *B. oleracea* varieties (*4*, *12*, *13*, *15*, *25*). Additionally, hexanal (**5**), allyl thiocyanate (**45**), allyl isothiocyanate (**46**), 3-butenyl isothiocyanate (**50**), 3-methylbutyl isothiocyanate (**52**), 4-methylpentyl isothiocyanate (**54**), 3-methylthiopropyl isothiocyanate (**56**), and phenylethyl isothiocyanate (**58**) were also already described in *B. oleracea* var. *botrytis* seeds (*15*, *17*).

Leaves were the material presenting the highest volatile content, β-ionone (**33**) being the major compound (**Table 1**). A number of biological activities have been described for this compound, namely, anticancer capacity (*26*), which may contribute to the known protective properties of kale. In addition, leaves exhibited the highest content of norisoprenoids (**Table 1**), which result from the oxidative cleavage of carotenoids (*27*). Carotenoids are tetraterpenoid pigments that are accumulated in the plastids of leaves, flowers, and fruits (*27*). This may explain the absence of norisoprenoids in the seeds and their reduced amounts in sprouts. In addition, norisoprenoid derivatives are important for the flavor of diverse food products (*28*).

Leaves also exhibited the highest content of aldehydes, alcohols, esters, and ketones (**Table 1**), which contribute to their green-leaf odor (*14*). With few exceptions, such as phenylacetaldehyde (**11**) and ethyl benzoate (**16**), which derive from amino acids (*27*), all of the identified aldehydes, alcohols, esters, and ketones are formed from fatty acids through a cascade of biochemical reactions (*27*). Only some of these compounds were detected in sprouts, and they were scarce in seeds. The rise in aldehydes, alcohols, ketones, and esters contents during kale development can be ascribed to the increased metabolic activity

Table 2. Glucosinolate Breakdown Products Identified in Kale and Their Precursors (*8*, *17*)

glucosinolate precursor	glucosinolate derivative
glucoputranjivin	isopropyl isothiocyanate (**44**)
sinigrin	allyl thiocyanate (**45**)
	allyl isothiocyanate (**46**)
sec-butyl glucosinolate	2-butyl isothiocyanate (**47**)
isobutyl glucosinolate	isobutyl isothiocyanate (**48**)
gluconapin	3-butenyl isothiocyanate (**50**)
n-butyl glucosinolate	*n*-butyl isothiocyanate (**51**)
3-methylbutyl glucosinolate	3-methylbutyl isothiocyanate (**52**)
pentyl glucosinolate	pentyl isothiocyanate (**53**)
4-methylpentyl glucosinolate	4-methylpentyl isothiocyanate (**54**)
hexyl glucosinolate	hexyl isothiocyanate (**55**)
glucoiberverin	3-methylthiopropyl isothiocyanate (**56**)
glucotropaeolin	benzyl thiocyanate (**57**)
gluconasturtiin	phenylethyl isothiocyanate (**58**)

of seeds, which rapidly resume the biochemical pathway above-mentioned, after germination (*27*, *29*).

Terpenes, an important group of compounds in leaves (**Table 1**), play an important role in the protection and reproduction of the plant; they have been described as toxins, repellents, or attractants to other organisms (*30*). In plants, terpenes are derived from the mevalonate (cytosol) or from the 2-*C*-methyl-D-erythritol-4-phosphate (plastids) pathways (*27*, *31*, *32*). It is generally recognized that the cytosolic pathway provides the precursors for sesquiterpene and triterpene production, whereas the precursors of isoprene, monoterpenes, diterpenes, and tetraterpenes are supplied by plastids (*27*, *31*, *32*). From all terpenic compounds identified in leaves, only some were found in the other materials. The small amount of these secondary metabolites in seeds and sprouts can be explained by the fact that, during germination, these matrices direct all available nutrients to primary metabolism (*33*), whereas in leaves secondary metabolism allows terpene formation.

Sulfur compounds are the main class of compounds in seeds and sprouts. This results from the contribution of a single compound, allyl isothiocyanate (**46**) (**Table 1**), a hydrolysis product of sinigrin, already described as the predominant glucosinolate in kale (*9*). However, the richness of seeds and sprouts in sulfur compounds also arises from the contribution of other glucosinolate degradation products, such as 2-butyl isothiocyanate (**47**), isobutyl isothiocyanate (**48**), 3-butenyl isothiocyanate (**50**), 3-methylbutyl isothiocyanate (**52**), 3-methylthiopropyl isothiocyanate (**56**), and phenylethyl isothiocyanate (**58**). The identified isothiocyanates allowed us to infer the presence of 14 glucosinolate precursors in kale materials (**Table 2**). All of them were already reported in *Brassica* (*2*, *3*, *8*, *9*, *11*, *17*). From those, gluconapin and gluconasturtiin were described in kale leaves (*7*), glucoiberverin was reported in its seeds (*34*), and sinigrin was reported in both materials (*9*). As far as we know, no studies revealed glucosinolates in kale sprouts.

Sulfur compounds (**43**−**58**) have important biological functions in plants and may also exert chemopreventive activity in humans (*11*). Nevertheless, numerous studies have demonstrated that glucosinolates exhibit outright toxicity and, consequently, isothiocyanates may contribute to the toxicity of their precursors (*23*). Due to their predominance, they most probably exert a relevant role in the characteristic aroma of seeds and sprouts. Dimethyl disulfide (**43**) and dimethyl trisulfide (**49**) derive from (+)-*S*-methyl-L-cysteine sulfoxide found in *Brassica* vegetables (*3*). However, sulfides can also be formed by subsequent degradation of some volatiles derived from glucosinolate breakdown (*3*).

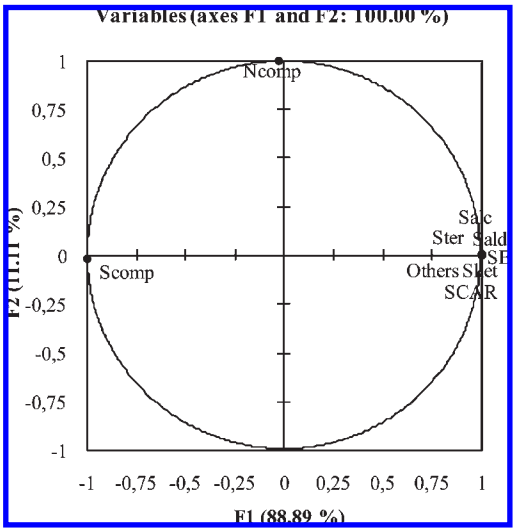

Figure 1. PCA of the volatile compounds in kale analyzed materials: projection of volatile compounds (variables: Salc, sum of alcohols; Sald, sum of aldehydes; SE, sum of ester compounds; Sket, sum of ketones; Ncomp, sum of nitrogen compounds; SCAR, sum of carotenoid molecules; Scomp, sulfur compounds; Ster, sum of terpenes; others, miscellaneous compounds) into the plane composed by the principal axes F1 and F2.

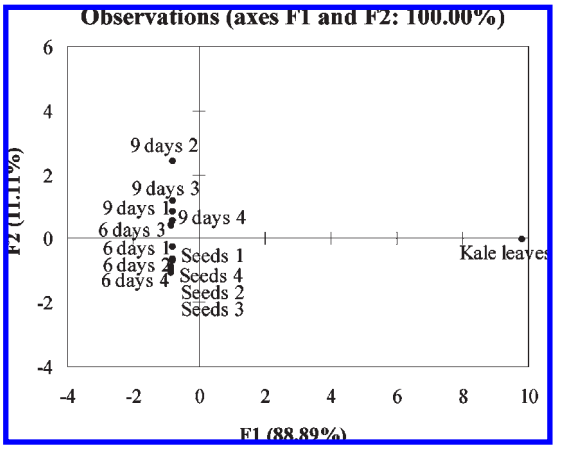

Figure 2. PCA of the volatile compounds in kale seeds, sprouts at 6 and 9 days, and leaves: projection of samples (seeds 1, seeds 2, seeds 3, seeds 4, 6 days 1, 6 days 2, 6 days 3, 6 days 4, 9 days 1, 9 days 2, 9 days 3 and 9 days 4) into the two principal components.

Statistical Analyses. To assess the variation of volatile composition during kale sprouting, PCA was performed on obtained data. **Figures 1** and **2** show the projection of chemical variables, grouped by families (sum of compounds of each chemical class), in all materials (seeds, sprouts, and leaves) into the plane composed by the principal axes F1 and F2 containing 100.00% of the total variance.

Concisely, kale leaves have a high positive correlation with all volatiles, except sulfur compounds. In contrast, seeds and sprouts are in very high correlation with sulfur compounds, which may reflect the need to maximize the defensive potential of these reproductive stages of the growth cycle, whereas kale leaves mostly use terpenic compounds as defense (*35*).

To get more information about seeds and sprouts, a similar PCA was performed without leaf samples. **Figures 3** and **4** show the projection of chemical variables, grouped by families (sum of compounds of each chemical class), in seeds and sprouts into the plane of F1 and F2 containing 88.15% of the total variance.

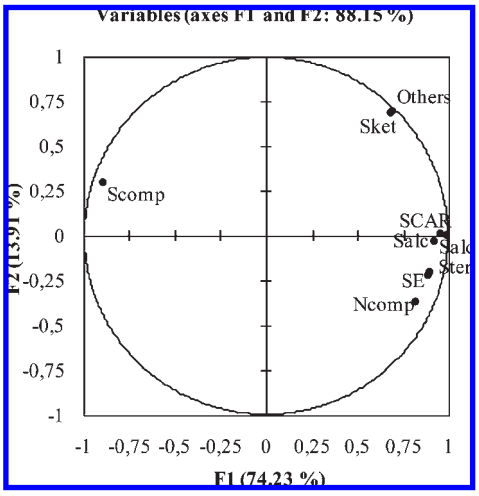

Figure 3. PCA of all the volatile compounds in kale seeds and sprouts: projection of volatile compounds (variables: Salc, sum of alcohols; Sald, sum of aldehydes; SE, sum of ester compounds; Sket, sum of ketones; Ncomp, sum of nitrogen compounds; SCAR, sum of carotenoid molecules; Scomp, sulfur compounds; Ster, sum of terpenes; others, miscellaneous compounds) into the plane composed by the principal axes F1 and F2.

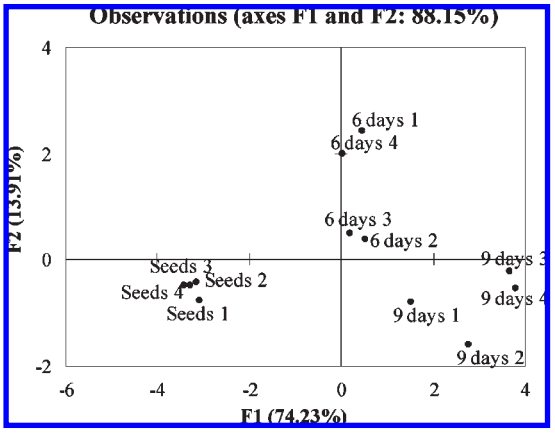

Figure 4. PCA of the volatile compounds in kale seeds and sprouts: projection of samples (seeds 1, seeds 2, seeds 3, seeds 4, 6 days 1, 6 days 2, 6 days 3, 6 days 4, 9 days 1, 9 days 2, 9 days 3, and 9 days 4) into the two principal components.

Sulfur compounds decrease during kale development, suggesting that these can be important constituents for the sprouting process. In addition, the transport of glucosinolates (precursors of (iso)thiocyanates and nitriles) from mature leaves to seeds, via phloem, was demonstrated (*23*). On the other hand, reproductive organs are also able to synthesize some of their own glucosinolates (*23*).

The divergent sulfur composition of seeds and the fact that the absolute amount of these compounds in this matrix is higher than that in the other stages of plant development also supports de novo synthesis of glucosinolates in these organs (*22*). With regard to volatile alcohols, aldehydes, esters, ketones, terpenes, and norisoprenoids formed during plant growth, reaching a maximum in fully developed leaves, it was shown that carbohydrates, fatty acids, and amino acids represent the natural carbon pools, which can also be liberated from their polymers (*27*, *36*). The higher absolute amount of sulfur compounds in seeds and the rise of alcohols, aldehydes, esters, ketones, terpenes, and norisoprenoids until the plant has fully matured have already been observed in other *Brassica* species (*12*, *13*, *15*, *25*).

In summary, as far as we know, the present study is the first report on the evolution of volatiles from seeds to mature plant. According to the results obtained, it may be anticipated that the volatile profile varies in accordance with the plant's development stage. In addition, kale's vegetal material, namely, seeds and sprouts, may have a wide biological potential, which include both negative (outright toxicity) and positive (chemoprotective effect against certain cancers) nutritional attributes. The rapid changes in glucosinolate profile that occur during germination and early seedling growth make the duration of the sprouting period to be a particularly relevant factor in maximizing the concentration of the desirable bioactive compounds. Therefore, the importance of the production and commercialization of *Brassica* sprouts is incremented.

LITERATURE CITED

(1) Sousa, C.; Lopes, G.; Pereira, D. M.; Taveira, M.; Valentão, P.; Seabra, R. M.; Pereira, J. A.; Baptista, P.; Ferreres, F.; Andrade, P. B. Screening of antioxidant compounds during sprouting of *Brassica oleracea* L. var. *costata* DC. *Comb. Chem. High Throughput Screen.* **2007**, *10*, 377–386.

(2) Bellostas, N.; Kachlicki, P.; Sørensen, J. C.; Sørensen, H. Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food. *Sci. Hortic.* **2007** (in press).

(3) Blažević, I.; Mastelić, J. Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.). *Food Chem.* **2009**, *113*, 96–102.

(4) Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. M.; Lipton, W. Additional volatile components of cabbage, broccoli, and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829–832.

(5) Wallbank, B. E.; Wheatley, G. A. Volatile constituents from cauliflower and other crucifers. *Phytochemistry* **1976**, *15*, 763−766.

(6) MacLeod, A. J.; Pikk, H. E. Volatile flavor components of fresh and preserved Brussels sprouts grown at different crop spacings. *J. Food Sci.* **1979**, *44*, 1183–1185.

(7) Velasco, P.; Cartea, M. E.; González, C.; Vilar, M.; Ordás, A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.* **2007**, *55*, 955–962.

(8) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5–51.

(9) Kushad, M. M.; Cloyd, R.; Babadoost, M. Distribution of glucosinolates in ornamental cabbage and kale cultivars. *Sci. Hortic.* **2004**, *101*, 215–221.

(10) Rosa, E. A. S.; Heaney, R. K.; Fenwick, G. R.; Portas, C. A. M. Glucosinolates in crop plants. *Hortic. Rev.* **1997**, *19*, 99–215.

(11) Wim, B. Y.; Jongen, M. F. Glucosinolates in *Brassica*: occurrence and significance as cancer-modulating agents. *Proc. Nutr. Soc.* **1996**, *55*, 433–446.

(12) Clapp, R. C.; Long, L.Jr.; Dateo, G. P.; Bisset, F. H.; Hasselstrom, T. The volatile isothiocyanates in fresh cabbage. *J. Am. Chem. Soc.* **1959**, *81*, 6278–6281.

(13) Bailey, S. D.; Bazinet, M. L.; Driscoll, J. L.; McCarthy, A. I. The volatile sulphur components of cabbage. *J. Food Sci.* **1961**, *26*, 163–170.

(14) Paré, P. W.; Tumlinson, J. H. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* **1999**, *121*, 325–331.

(15) Valette, L.; Fernandez, X.; Poulain, S.; Loiseau, A.-M.; Lizzani-Cuvelier, L.; Levieil, R.; Restier, L. Volatile constituents from Romanesco cauliflower. *Food Chem.* **2003**, *80*, 353–358.

(16) Kondo, H.; Nozaki, H.; Hawaguchi, T.; Naoshima, Y.; Hayashi, S. The effect of germination upon the volatile components of rape seeds (*Brassica napus* L.). *Agric. Biol. Chem.* **1984**, *48*, 1891–1893.

(17) Valette, L.; Fernandez, X.; Poulain, S.; Lizzani-Cuvelier, L.; Loiseau, A.-M. Chemical composition of the volatile extracts from

Brassica oleracea L. var. *botrytis* 'Romanesco' cauliflower seeds. *Flavour Fragrance J*. **2006**, *21*, 107–110.
(18) Zhao, D.; Tang, J.; Ding, X. Analysis of volatile components during potherb mustard (*Brassica juncea*, Coss.) pickle fermentation using SPME-GC-MS. *LWT−Food Sci. Technol*. **2007**, *40*, 439–447.
(19) Guedes de Pinho, P.; Ribeiro, B.; Gonçalves, R. F.; Baptista, P.; Valentão, P.; Seabra, R. M.; Andrade, P. B. Correlation between the pattern volatiles and the overall aroma of wild edible mushrooms. *J. Agric. Food Chem*. **2008**, *56*, 1704–1712.
(20) Guedes de Pinho, P.; Gonçalves, R. F.; Valentão, P.; Pereira, D. M.; Seabra, R. M.; Andrade, P. B.; Sottomayor, M. Volatile composition of *Catharanthus roseus* (L.) G. Don using solid-phase microextraction and gas chromatography mass spectrometry. *J. Pharm. Biomed. Anal*. **2009**, *49*, 674–685.
(21) Di, X.; Shellie, R. A.; Marriot, P. J.; Huie, C. W. Application of headspace solid-phase microextraction (HS-SPME) and comprehensive two-dimensional gas chromatography (GC × GC) for the chemical profiling of volatile oils in complex herbal mixtures. *J. Sep. Sci*. **2004**, *27*, 451–458.
(22) Brown, P. D.; Tokuhisa, J. G.; Reichelt, M.; Gershenzon, J. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* **2003**, *62*, 471–481.
(23) Halkier, B. A.; Gershenzon, J. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol*. **2006**, *57*, 303–333.
(24) Mithen, R. Glucosinolates—biochemistry, genetics and biological activity. *Plant Growth Regul*. **2001**, *34*, 91–103.
(25) MacLeod, A. J.; MacLeod, G. Volatiles of cooked cabbage. *J. Sci. Food Agric*. **1968**, *19*, 273–277.
(26) Liu, J.-R.; Sun, X.-R.; Dong, H.-W.; Sun, C.-H.; Sun, W.-G.; Chen, B.-Q.; Song, Y.-Q.; Yang, B.-F. β-ionone suppresses mammary carcinogenesis, proliferative activity and induces apoptosis in the mammary gland of the Sprague−Dawley rat. *Int. J. Cancer* **2008**, *122*, 2689–2698.
(27) Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavour compounds. *Plant J*. **2008**, *54*, 712–732.
(28) Goff, S. A.; Klee, H. J. Plant volatile compounds: sensory cues for health and nutritional value?. *Science* **2006**, *311*, 815–819.
(29) Pracharoenwattana, I.; Cornah, J. E.; Smith, S. M. Arabidopsis peroxisomal citrate synthase is required for fatty acid respiration and seed germination. *Plant Cell* **2005**, *17*, 2037–2048.
(30) Gershenzon, J.; Dudareva, N. The function of terpene natural products in the natural world. *Nat. Chem. Biol*. **2007**, *3*, 408–414.
(31) Dudareva, N.; Pichersky, E.; Gershenzon, J. Biochemistry of plant volatiles. *Plant Physiol*. **2004**, *135*, 1893–1902.
(32) Aharoni, A.; Jongsma, M. A.; Bouwmeester, H. J. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci*. **2005**, *10*, 594–602.
(33) Bellani, L. M.; Guarnieri, M.; Scialabba, A. Differences in the activity and distribution of peroxidases from three different portions of germinating *Brassica oleracea* seeds. *Physiol. Plant*. **2002**, *114*, 102–108.
(34) West, L. G.; Meyer, K. A.; Balch, B. A.; Rossi, F. J.; Schultz, M. R.; Haas, G. W. Glucoraphanin and 4-hydroxyglucobrassicin contents in seeds of 59 cultivars of broccoli, raab, kohlrabi, radish, cauliflower, brussels sprouts, kale, and cabbage. *J. Agric. Food Chem*. **2004**, *52*, 916–926.
(35) Howe, G. A.; Jander, G. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol*. **2008**, *59*, 41–66.
(36) Croteau, R.; Karp, F. Origin of natural odorants. In *Perfumes. Art, Science and Technology*; Muller, P. M., Lamparsky, D., Eds.; Elsevier Applied Science: London, U.K., 1991; pp 101−126.

Received March 9, 2009. Revised manuscript received June 29, 2009. Accepted July 4, 2009. We acknowledge Fundação para a Ciência e Tecnologia (FCT) for financial support (PTDC/AGR-AAM/64150/2006). F.F. is grateful to FCT for a grant (SFRH/BD/37963/2007).