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Volatile constituents from Romanesco cauliflower

L. Valette[a], X. Fernandez[a,*], S. Poulain[a,*], A.-M. Loiseau[a],
L. Lizzani-Cuvelier[a], R. Levieil[b], L. Restier[c]

[a]*Laboratoire Arômes, Synthèses et Interactions, Faculté des Sciences de Nice-Sophia Antipolis,
Parc Valrose, 06108 Nice cedex 2, France*
[b]*CLAUSE, Station de Recherche Nord, 1 chemin du Moulin des Ronzières, 49800 La Bohalle, France*
[c]*CLAUSE, Le Mas Saint-Pierre, La Galine, 13210 Saint Rémy de Provence, France*

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Abstract

The volatile and semi-volatile constituents of *Brassica oleracea* L. var. *Botrytis* L., Romanesco group, Navona variety, were extracted by steam distillation from leaves and inflorescence, using a Likens–Nickerson-type apparatus. The extracts from fresh, ripening and frozen vegetables were investigated by gas chromatography and mass spectrometry. From the fresh leaves extract, a total of 61 compounds were identified, representing 96.8% of the oil. The major constituent was found to be hex-3(Z)-enol (61.1%). From fresh disrupted inflorescence tissues of Romanesco, 35 compounds were detected, representing 97.7% of the extract. Dimethyl disulfide, dimethyl trisulfide and hex-3(Z)-enol were identified as major constituents of the hydrodistillation products, representing, respectively, 30.2, 24.2 and 21.7% of the volatiles. From ripening and frozen inflorescence tissues, dimethyl disulfide and trisulfide were again detected as predominant components. In the latter, hex-3(Z)-enol had almost disappeared (0.8%) whereas dimethyl trisulfide represented 49.7% of the oil. This is the first report concerning the steam distillation compounds present in the leaves and inflorescence tissues of this species.

Keywords: *Brassica oleracea* L. var. *Botrytis* L.; Romanesco cauliflower; Likens–Nickerson-type apparatus; Volatiles; Sulfur compounds; Alkyl isothiocyanates; Dimethyl disulfide; Dimethyl trisulfide; Hex-3(Z)-enol

1. Introduction

Since the end of the sixties, much work has been carried out on the nature of the flavour volatiles of foodstuffs, using a combination of capillary gas chromatography and mass spectrometry. Cruciferous vegetables are known to contain organic sulfur. Their characteristic flavour and odour have been attributed to volatile sulfur containing compounds. Many studies have been reported, concerning cabbages, cauliflowers, Brussels sprouts and broccoli (Buttery, Guadagni, Ling, Seifert, & Lipton, 1976; Daxenbichler, Van Etten, & Spencer, 1977; MacLeod & MacLeod, 1970c; Marks, Hilson, Leichtweis, & Stoewsand, 1992; Pearson, Dawson, & Lackey, 1981; Van Langenhove, Cornelisc, & Schamp, 1991; Wallbank & Wheatley, 1976). Composition of volatile constituents has been used to identify new volatile flavouring compounds, such as sulfides or isothiocyanates (Maruyama, 1970), to evaluate the effects of variations in cooking methods (MacLeod & MacLeod, 1970a) and to compare the volatile fractions from various cruciferous vegetables (MacLeod & MacLeod, 1970b). Studies to improve the storage time of cruciferous vegetables (MacLeod & Pikk, 1979) have emphasised the need for an improved knowledge of the compounds contributing to their flavor.

Much work has been carried out on raw material. Some vegetables have been investigated, to analyse preservation effects on volatile compound composition. MacLeod et al. (1970c) reported analytical data on flavour volatile composition of a number of dehydrated cabbage samples, indicating the poor nature of the preserved product as a substitute for the fresh vegetable. Using the same sampling techniques (MacLeod et al.,

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* Corresponding authors. Tel.: +33-492-07-65-77; fax: +33-492-07-61-25.

E-mail addresses: xfernand@unice.fr (X. Fernandez), poulain@unice.fr (S. Poulain).

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1970b), the volatile constituents of cooked vegetables were collected and analysed by gas chromatography, in order to compare them with the fresh material. MacLeod et al. (1979) have also examined the effect of freezing and blanching on the nature of the volatile flavour components of Brussels sprouts: appreciable variations in the chemical composition of the flavour extracts were observed, and possible reasons for unusual and unacceptably strong flavours of frozen Brussels sprouts were suggested.

In most of these studies, the vegetables were placed in boiling water. Volatile compounds were water-distillated and collected at low temperature (Maruyama, 1970; MacLeod & MacLeod, 1968, 1970a, 1970b, 1970c, 1979), trapped on an adsorption tube (Van Langenhove et al., 1991) or extracted with solvents (Daxenbichler et al., 1977). Very few studies deal with other techniques, such as hydrodistillation using a Likens–Nickerson-type apparatus (Buttery et al., 1976) or static and dynamic headspace analysis (Wallbank et al., 1976). In each case, sulfides, polysulfides, thiols, isothiocyanates, nitriles, alcohols, carbonyl compounds (ketones, aldehydes, esters), furans and terpene hydrocarbones were identified in cabbage, cauliflower, Brussels sprouts and broccoli. Dimethyl trisulfide was present as a major aroma component in cooked *Brassicaceous* vegetables (Maruyama, 1970). Moreover, isothiocyanates and nitriles were also found to be predominant in Brussels sprouts, whereas aldehydes were the most abundant cauliflower volatiles (Van Langenhove et al., 1991). For cabbage, evidence based on GC retention times was obtained by Buttery et al. (1976), 2,3,5-trithiahexane, dimethyl sulfide, dimethyl trisulfide, 2-phenylethyl cyanide and 2-phenylethyl isothiocyanate were identified as important aroma components.

Much less interest was devoted to Romanesco cauliflower, as these species have been commercialised for less than 3 years. This new cauliflower variety has a unique flavour and an atypical green and pyramidal inflorescence, so it is well appreciated by consumers. No spectral evidence has been previously obtained to analyse volatiles isolated from the leaves. Evidently only one study concerns volatile extraction from Romanesco cauliflower (Di Cesare, Fusari, Maestrelli, Avitabile Leva, & Ferrari, 2001): volatile compounds from raw and cooked florets were extracted by microwave and analysed by GC–MS. The compounds identified in the extract and their importance were described: dimethyl trisulfide, dimethyl tetrasulfide and methyl isothiocyanates were reported as main volatile constituents in the raw extract, whereas thionitriles and nitriles were prevalent in cooked florets. However, no identification of specific components was carried out in the leaves.

In the present paper, investigations of volatile compounds from the leaves and the inflorescence of fresh, ripening or frozen species of *Brassica oleracea* L. var. *Botrytis* L., *Romanesco* group are described for the first time.

2. Materials and methods

2.1. Materials

Romanesco cauliflowers, *Brassica oleracea* L. var. *Botrytis* L., were obtained from a seed trader (CLAUSE, Saint Rémy de Provence, France). The studied species is commercialised under the name "Romanesco Navona". All cauliflower samples were cut at the same time. One set was analysed immediately; one set was stored for 17 days at 20 °C and one set was frozen at −20 °C until analysed. Botanical identification of the vegetable was conducted by Rémi Levieil, an agricultural engineer from CLAUSE company.

2.2. Methods

Standard conditions were used for all experiments. Fresh leaves (450 g) were cut into small pieces and placed in a 2-l flask with 0.8 l of water. The mixture was subjected to hydrodistillation using a Likens–Nikerson-type apparatus. The volatile fraction was extracted under atmospheric pressure during 5 h, using dichloromethane (50 ml) as extracting solvent. This extract was dried over anhydrous sodium sulphate and carefully concentrated to small volume (~0.5 ml), with a rotary evaporator at atmospheric pressure. Further, this oil was stored at low temperature prior to analysis. The fresh, ripening and frozen inflorescences were steam-distilled, following the method previously described. The frozen vegetables were first thawed using a microwave oven during 10 min (defrozen mode). Before analysis, the extracts were treated as indicated above.

2.3. Capillary GC–mass spectral analysis

Each concentrated extract sample was analysed by GC–MS, using a Hewlett-Packard 5890/5970A system, with a HP1 column (50 m×0.20 mm fused silica capillary column; film thickness, 0.5 μm). GC oven initial temperature was 60 °C and was programmed to 220 °C at a rate of 2 °C/min, and finally held at 220 °C during 40 min. Operating conditions of the GC were as follows: helium was used as carrier gas (0.8 ml/min); the temperature of injector and detector was 250 °C; the volume injected was 0.4 μl (dichloromethane solution) in splitless mode. Retention indices were determined with C_5–C_{28} alkane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35–400. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component identified (Table 1).

Table 1
Volatile components of the Romanesco cauliflower (Navona variety)

Components[a]	RI[b]	Leaves (% ± S.D.[c])	Inflorescence (% ± S.D.[c])		
		Fresh	Fresh	Ripening	Frozen
Pyridine[d]	712	0.1	0.2 ± 0.1	–	0.3
Dimethyl disulfide	722	2.1 ± 0.2	30.2 ± 2.5	22.3 ± 1.2	21.8 ± 0.5
Pent-2(Z)-enol	740	3.6 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Hexanal[d]	770	0.5	0.5 ± 0.1	0.3	1.1 ± 0.1
Dimethyl sulfoxide[d]	772	–	0.2 ± 0.1	–	–
Octane	792	–	0.2	–	–
Furfural[d]	796	1.5	0.7 ± 0.1	2.3 ± 0.2	1.4 ± 0.2
Hex-2(E)-enal	823	1.9 ± 0.2	1.0 ± 0.2	1.7 ± 0.1	6.0 ± 0.3
Hex-3(Z)-enol	835	61.1 ± 2.0	21.7 ± 1.0	8.6 ± 0.1	0.8 ± 0.2
Hex-2(E)-enol	840	1.4 ± 0.2	–	0.8 ± 0.1	–
Hexan-1-ol	843	1.1 ± 0.1	1.8 ± 0.1	0.6 ± 0.1	tr
3-(methylthio)propanal[d]	862	0.1	0.2	0.3	0.4 ± 0.1
Heptanal[d]	873	–	0.3 ± 0.1	0.1	0.1
2-Acetylfuran[d]	876	tr	–	–	–
2,6-Dimethylpyrazine	882	–	–	0.1	0.2
Methylpentyl sulfide	902	–	–	–	tr
i-Butyl isothiocyanate	919	–	0.1	–	–
α-Pinene[d]	932	–	–	–	0.1
Dimethyl trisulfide	953	6.5 ± 0.5	24.2 ± 1.5	42.1 ± 1.5	49.7 ± 3.2
Sabinene[d]	966	–	–	–	0.2
β-Pinene[d]	971	–	–	–	0.3
2-Pentylfuran	973	0.1	tr	0.2	0.1
Hepta-2(E),4(E)-dienal	974	0.3 ± 0.1	0.2 ± 0.1	0.1	0.3
2-Acetylthiazole[d]	976	–	tr	0.1	–
Hex-3(Z)-enyl acetate	979	4.1 ± 0.3	–	–	–
2-(Pent-2(E)-enyl)furan	980	0.3	0.1	0.2	0.2
Hexyl acetate[d]	986	0.1	–	–	–
Phenylacetaldehyde	1004	0.9 ± 0.1	0.1	1.7 ± 0.2	0.8 ± 0.2
2,2,6-Trimethylcyclohexanone	1011	0.1	–	–	–
Limonene[d]	1017	–	–	–	0.1
Unknown (1)[e]	1023	0.2	0.9 ± 0.2	0.2 ± 0.1	0.1
3-(Methylthio)propyl cyanide	1027	0.1	0.4 ± 0.1	2.2 ± 0.1	2.9 ± 0.2
1-(Methylthio)pentan-3-one	1045	–	tr	0.2	0.2
γ-Terpinene[d]	1046	0.1	–	–	–
Octa-3(E),5(E)-dien-2-one	1057	–	tr	0.1	tr
Nonanal[d]	1075	0.3 ± 0.1	2.2 ± 0.2	1.0 ± 0.1	0.7
Phenylacetonitrile	1084	tr	–	tr	tr
2,3,5-Trithiahexane	1094	0.1	1.0 ± 0.1	2.0 ± 0.2	1.5 ± 0.1
Nona-2(E),6(Z)-dienal	1117	0.1	–	tr	tr
Camphor[d]	1120	0.1	tr	–	–
3-Ethylphenol	1130	–	–	0.1	–
Pinocarvone[d]	1137	0.1	–	–	–
4-(Methylthio)butyl cyanide	1142	0.1	0.4 ± 0.1	3.7 ± 0.4	1.7 ± 0.1
Thujen-2-one	1144	tr	–	–	–
Terpinen-4-ol[d]	1157	–	–	–	tr
Hex-3(Z)-enyl butanoate	1159	0.4 ± 0.1	–	–	–
α-Terpineol[d]	1169	tr	–	–	–
Safranal[d]	1172	tr	–	–	–
4-Vinylphenol	1173	–	–	tr	tr
Decanal	1177	tr	tr	tr	tr
Dodec-1-ene	1180	0.1	–	–	–
2-Phenylethyl cyanide	1183	tr	tr	–	–
Dimethyl tetrasulfide	1187	0.2 ± 0.1	0.5 ± 0.2	2.9 ± 0.3	3.6 ± 0.1
β-Cyclocitral[d]	1194	0.4 ± 0.1	–	–	–
β-Cyclohomocitral	1235	0.1	–	–	–
Unknown (2)[f]	1239	1.0 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Indole	1248	1.2 ± 0.1	0.1	0.9 ± 0.1	0.7 ± 0.1
3-(Methylthio)propyl isothiocyanate	1260	0.5 ± 0.1	3.6 ± 0.3	0.3 ± 0.1	0.1
p-Vinylguaiacol[d]	1277	1.0	tr	0.4 ± 0.1	0.2
Tridec-1-ene	1281	0.4	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Components[a]	RI[b]	Leaves (% ± S.D.[c])	Inflorescence (% ± S.D.[c])		
		Fresh	Fresh	Ripening	Frozen
Deca-2(E),4(E)-dienal	1282	0.1	tr	tr	tr
Tridecane	1292	0.1	–	–	–
Eugenol[d]	1319	–	–	tr	–
1,1,6-Trimethyl-1,2-dihydronaphtalene	1336	tr	–	tr	–
3-Methylindole	1341	–	–	tr	tr
β-Damascenone[d]	1358	0.1	–	tr	–
1-Mesitylbuta-1,3-diene	1373	0.2	–	–	–
4-(Methylthio)butyl isothiocyanate	1383	0.6±0.1	5.6±0.4	0.7±0.1	0.4±0.1
β-Damascone	1392	0.2	–	–	–
2-Phenylethyl isothiocyanate	1419	0.2	0.6±0.1	0.1	tr
Trans-β-ionone-5,6-epoxyde	1460	0.1	–	–	–
Trans-β-ionone[d]	1461	0.4±0.1	–	tr	tr
Tridecanal	1484	0.1	–	–	–
2,6-Dimethyl-3-(methoxymethyl)-*p*-benzoquinone	1516	tr	–	0.2	tr
Hex-3(Z)-enyl benzoate	1542	tr	–	–	–
Tetradecanal	1586	1.6±0.2	–	tr	–
Pentadecan-2-one	1673	tr	–	tr	–
Pentadecanal	1690	1.9±0.2	–	tr	–
Benzyl benzoate[d]	1729	tr	–	–	–
Hexadecan-2-one	1778	0.1	–	–	–
Methyl palmitate	1904	tr	–	tr	–
Spectra found (%)		96.8	97.7	97.4	97.2
Identified components[g]		61	35	45	42
Sulfur-containing components (%)		10 (10.5)	14 (67.0)	12 (76.9)	12 (82.3)
Isothiocyanates (%)		3 (1.3)	4 (9.9)	3 (1.1)	3 (0.5)
Cyanides (%)		4 (0.2)	3 (0.8)	3 (5.9)	3 (4.6)
Alcohols (%)		6 (68.2)	4 (25.1)	8 (11.6)	6 (2.3)
Aldehydes (%)		16 (9.8)	10 (5.2)	13 (7.5)	11 (10.8)

[a] Compounds are listed in order of their elution time from a HP1 column. Compositional values less than 0.1% are denoted as traces (tr).

[b] RI = retention indices as determined on HP1 using the homologous series of *n*-alkanes.

[c] S.D. = standard deviation.

[d] Structure confirmed by standard compound injection.

[e] Unknown (1): 129(M$^+$, 100), 114(43.5), 100(17.3), 73(48.7), 72(58.0), 71(26.5), 70(14.9), 57(60.0), 55(30.5), 43(70.3), 41(85.5), 39(31.2).

[f] Unknown (2): 147(M$^+$, 100), 132(72.6), 118(23.3), 117(31.8), 116(91.3), 90(16.0), 89(47.4), 77(33.0), 63(22.3), 62(10.3), 51(11.4), 39(10.6).

[g] Unidentified components less than 0.5% are not reported.

3. Results and discussion

Volatile compounds present in trace amounts may contribute significantly to the characteristic flavour and aroma of fresh, ripening or cooked foods. This investigation was performed to identify the chemical structure of important aroma components in Romanesco cauliflower. The volatile fraction was investigated by a GC–MS combination. All the constituents were identified by comparison of their mass spectra with those stored in MS databases (Wiley 6N; MassFinder 2.1 Library), with literature data (Adams, 1995; Joulain & König, 1998; Joulain, König, & Hochmuth, 2001; McLafferty & Stauffer, 1989), and with home-made mass spectra libraries (built up from pure substances and MS literature data). Identification was confirmed by comparison of their retention indices (RI) with those of authentic samples available in the authors' laboratory, or with GC data previously published (Davies, 1990; ESO 2000, 1999). The components of the Romanesco cauliflower extracts, the percentage of each constituent and the retention indices are given in Table 1: compounds are listed in order of their elution on the HP-1 column.

The chromatographic profile of fresh leaves oil reveals that it contains 61 constituents, representing 96.8% of the extract. The studied sample was dominated by the unsaturated alcohol fraction that amounted to 67.1% of the oil. In particular, hex-3(Z)-enol (61.1%) was found to be the major constituent of the extract. Other minor compounds were observed, such as dimethyl trisulfide (6.5%) and dimethyl disulfide (2.1%), although their proportions were insignificant compared with the alcohols.

The same experiment was conducted with fresh inflorescence. A total of 35 volatile constituents were detected in the sample oil, representing 97.7% of the extract. The volatiles of inflorescence were abundant in sulfide compounds, more exactly in dimethyl disulfide (30.2%), dimethyl trisulfide (24.2%) and dimethyl tetrasulfide (0.5%). It is noteworthy that four alkyl isothiocyanates (0.1–5.6%) were also identified, according to the literature, and their mass spectra data. In addition hex-3(Z)-enol was detected among the volatile constituents of the studied sample, but to a smaller extent (21.7%) compared to the leaves (61.1%).

Comparing the chromatographic profiles obtained with fresh leaves and fresh inflorescence, an interesting fact is noteworthy: the sulfide fraction is predominant in Romanesco cauliflower inflorescence, whereas unsaturated alcohols are the major components of the leaves of this vegetable. This has already been reported by MacLeod et al. (1970a, 1970b): the sulfur volatiles are rather produced by the young part of the cabbage whereas the older leaves liberate a greater proportion of alcohols, aldehydes and ketones. Most of the volatiles liberated from the cauliflower (*Brassica* family) are produced by the inflorescence rather than the outer leaves.

Ripening and frozen inflorescence were investigated under the same conditions. A total of 45 and 42 compounds were detected in each of these extracts, representing, respectively, 97.4 and 97.2% of the volatiles. These two samples were dominated by the sulfide and the cyanide fractions, representing, respectively, 69.3 and 5.9% of the oil in the ripening vegetable, and 76.6% and 4.6% in the frozen inflorescence. Dimethyl trisulfide has already been reported as the major aroma component in cooked Brassicaceous vegetables by Maruyama (1970). The flavour profile of fresh cauliflower is quite different from that of the preserved vegetable. The decrease or the complete loss of unsaturated alcohols and aldehydes, observed in ripening and frozen vegetables, is compensated by a huge increase in polysulfide volatiles. The increased amount of such compounds probably contributes to the characteristic cauliflower flavour, distinguishing the fresh vegetable from the ripening one. An important variation concerns the ratio of alkyl isothiocyanates produced. It is considerably reduced in frozen cauliflower, whereas the alkyl cyanide amount is noticeably increased. Glucosinolate decomposition leads to the formation of isothiocyanates or cyanides, MacLeod (1970c) suggested that glucosinolates are decomposed enzymatically, to give isothiocyanates and thermally, to afford the cyanides. The decrease in isothiocyanate ratio is probably due to the inactivation of the enzyme myrosinase during the preservation stage. The increased amount of cyanides is most likely the result of a thermal decomposition of the sinigrin during the processing. The main differences observed between fresh and frozen Romanesco cauliflower are in good agreement with those already described by MacLeod et al. (1970b) in *Brassica oleracea* L. var. *gemmifera* L. and var. *Botrytis* L.

Most of the volatiles identified in Romanesco cauliflower from southern France have not been previously described by Di Cesare et al. (2001). These include hex-3(Z)-enol, 2,3,5-trithiahexane, 3-(methylthio)propyl cyanide, 3-(methylthio)propyl isothiocyanate, 4-(methylthio) butyl isothiocyanate and 2-phenylethyl isothiocyanate, which have already been reported in other vegetables (cabbage and cauliflower, Buttery et al., 1976). Chromatographic profiles revealed the presence of several compounds in small quantities ($\leqslant 0.5\%$). Some investigations are now in progress to identify the chemical structure of these aroma components. A combination of flame-ionisation (FID) and flame photometric detector (FPD) detection methods will be used to confirm the presence of sulfur in these molecules. Synthesis of several families of sulfur-containing compounds will be considered, such as isothiocyanates, cyanides or heterocycles. These results are the starting point of a new topic in our laboratory: it is well known that the characteristic flavours and odours of many foods have been attributed to some of these sulfur-containing volatile components. The synthesis developed in our team could provide spectral data of trace level components and could allow the estimation of the importance of these molecules in food aroma. We are also interested in other extracting methods, such as solid phase microextraction (SPME), which requires no solvent and could be very useful for analysis of trace level volatile compounds in natural products. These experiments are still under investigation in our laboratory and should be the subject of a forthcoming publication.

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