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Flavour profile of capers (*Capparis spinosa* L.) from the Eolian Archipelago by HS-SPME/GC–MS

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Abstract

This paper presents the first investigation of the flavour profile of capers (*Capparis spinosa* L.) from the Eolian Archipelago. In all, 145 volatile compounds were identified and quantified in capers, by HS-SPME/GC–MS analysis. Aldehydes (22.2%) and esters (21%) were the most abundant chemical classes; five sesquiterpenes and ten monoterpenes were identified for the first time; among sulphur compounds (8.42%), methyl-isothiocyanate was the major one, followed by benzyl-isothiocyanate. The application of this solvent-free extraction technique combined with the GC–MS analysis, showed its potential as a simple routine method for analyzing food flavour.

Keywords: HS-SPME/GC–MS; Capers; Flavour profile

1. Introduction

Capparis spinosa L. (Capparaceae) is a common perennial shrub in the Mediterranean regions, growing both wild and cultivated, with medicinal and aromatic properties. Although its ancient habitat is thought to be the dry areas of western or central Asia, the plant has a natural distribution in the coastal regions of the entire Mediterranean Sea basin; its range stretches from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran.

The first recorded use of *C. spinosa* was for medicinal purposes in 2000 BC by the Sumerians. The ancient Greeks and Romans also used the plant for these purposes. The fruits and the root of the plant have been used in gout and also as diuretics, astringents and tonics in traditional Iranian medicine (Afsharypuor, Jeiran, & Jazy, 1998). Even its flower buds have some medical uses and are taken to improve liver functions or as a kidney disinfectant. Moreover, it was reported that the plant possesses significant anti-inflammatory activity against carrageenan-induced edema in rats (Al-Said, Abdelsattar, Khalifa, & El-Feraly, 1988).

C. spinosa L. is one of the most commonly found aromatic plants in Mediterranean cooking: the fresh aerial parts, including the fruit and the flower buds, are stored in vinegar or brined and eaten pickled (Zargari, 1986). The floral buds of this plant are commonly named capers; they are harvested in spring before they blossom and are usually processed in brine. The processed buds have long been used in recipes for salads, pasta, meat, sauces and garnishes to add a pungent spicy flavour and aroma to food and have gained a considerable importance in the food industry. Spain is the leading world producer of capers followed by Morocco, Italy and Turkey, respectively. In Italy, they are mainly cultivated in the Eolian Archipelago (1400 quintal/year) and in the Mediterranean island of Pantelleria (404 quintal/year), where they have become an important economic crop.

We previously reported (Giuffrida, Salvo, Ziino, Toscano, & Dugo, 2002) on some chemical constituents of

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capers from the island of Salina, and now we are studying their flavour profile. There is a very limited number of reports on caper flavour; in fact, the investigation of the flavour profile of capers from Morocco was reported by Brevard, Brambille, Chaintreau, and Marion (1992), and that of capers from Iran was reported by Afsharypuor et al. (1998), which both used distillation-based, volatile extraction techniques. Recently, emphasis has been placed on developing solvent-free sample preparation methods, while flavour study on different food has been of increasing interest due to its relationship with the quality of food products. In the present study, Head Space Solid-Phase Microextraction (HS-SPME) was used, as a solvent-free sample preparation method, with gas chromatography–mass spectrometry (GC–MS) analysis, to provide the initial investigation of the flavour profile of capers from the Eolian Archipelago.

2. Materials and methods

2.1. Samples

Pickled caper samples were purchased from four local farmers on the island of Salina (Eolian Archipelago, Sicily) in August 2004. The usual commercial processing procedure for preparing pickled capers in Salina consists in a pre-treatment in which raw capers are mixed with marine salt (25% by weight) for about ten days; during this time a brine is formed and fermentation takes place. At the end of this stage the brine is discarded and the capers are treated with marine salt (15% by weight) for another ten days obtaining the finished product.

Twenty-four samples were analyzed; two different samples from each farmer, each representative of a 100 kg production, were collected after 30 days of storage and analyzed in triplicate.

2.2. HS-SPME Procedure

The extraction of volatile compounds was carried out by a HS-SPME (headspace solid phase microextraction) method using a DVB/CAR/PDMS fiber, with 50/30 μm film thickness (Supelco, Bellafonte, PA, USA); before the analysis the fiber was preconditioned in the injection port of the GC as indicated by the manufacturer.

For each sample 6 g of capers, previously homogenized, were weighed into a 40 ml vial and suspended in 14.5 ml; the vial was equipped with a ''mininert'' valve (Supelco, Bellafonte, PA, USA). The vial was kept at 35 °C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35 °C. After sampling the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption.

In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time, were

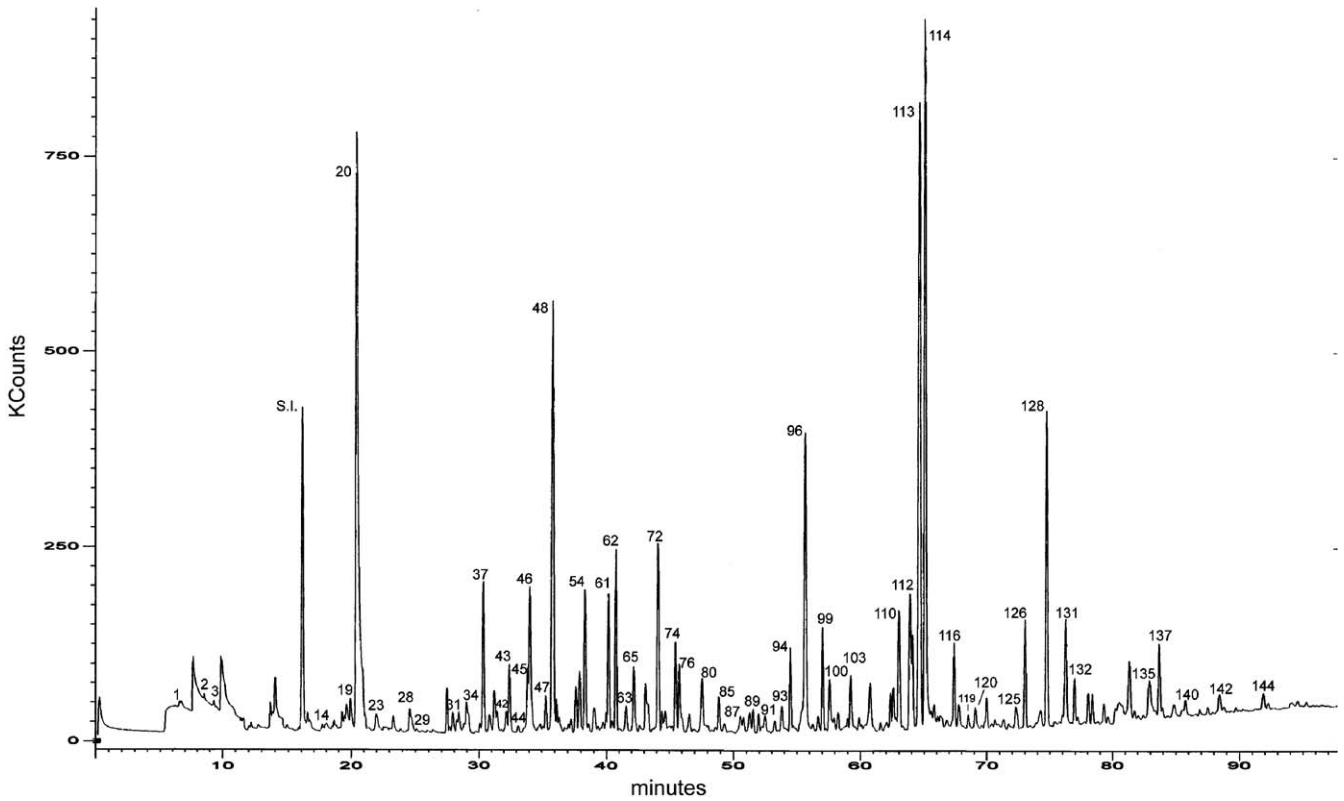


Fig. 1. GC–MS chromatogram of capers (*Capparis spinosa* L.) from the Eolian Archipelago extracted by HS-SPME.

studied on the extraction efficiency as previously reported by Verzera, Ziino, Condurso, Romeo, and Zappalà (2004). Each measurement was repeated three times. The repeatability of the developed method was determined by analysing three different samples of the same caper batch under identical experimental conditions; the criteria of the efficiency was the desorption peak area (total ion chromatogram) and the coefficient of variation (CV%) of the measurements.

Table 1
Acids (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Acids	LRI[b]	R[c]	X[d]	SD[e]
39	Acetic Acid	1450	A, B, C, D	32.48	2.09
93	Hexanoic acid	1841	A, B, C, D	31.03	2.37
104	Heptanoic acid	1946	A, B, C	9.65	0.60
114	Octanoic acid	2053	A, B, C, D	730.05	62.01
129	*N*-decanoic acid	2263	A, B, C	4.80	0.72
135	Benzoic acid	2433	A, B, C, D	64.28	3.10
146	Tetradecanoic acid	2698	A, B, C	9.13	0.95

[a] Number of peaks.
[b] LRI: linear retention indices calculated for a CP-Wax 52 CB column.
[c] R: reliability of identification; A, tentative identification by mass spectrum; B, authentic standard; C, linear retention indices from the literature; D, compounds identified by Brevard et al.
[d] *X*: mean value of eight samples, each sample in triplicate.
[e] SD: standard deviation.

Table 2
Esters (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Esters	LRI[b]	R[c]	X[d]	SD[e]
5	Ethyl butanoate	1034	A, B, C	0.34	0.08
8	2-Methylbutyl-acetate	1119	A, C	0.32	0.08
17	Butyl butanoate	1218	A, C	14.98	0.78
22	Hexyl acetate	1272	A, B, C, D	13.40	2.83
26	Methyl heptanoate	1296	A, C	5.52	1.03
31	Methyl octanoate	1390	A, B, C	14.65	1.46
35	Butyl hexanoate	1413	A, C	18.59	1.21
37	Ethyl octanoate	1434	A, B, C	149.01	20.18
41	3-Ethylbutyl hexanoate C6 isopentile	1459	A	5.39	1.34
62	Butyl-octanoate	1613	A, C	128.62	7.42
63	Methyl Benzoate	1628	A, B	23.26	1.64
64	Ethyl decanoate	1638	A, B, C	16.32	3.69
70	Isopentyl octanoate	1658	A	46.86	10.45
72	Ethyl benzoate	1670	A, B, D	138.61	8.88
80	Benzyl acetate	1731	A, B, D	34.81	2.08
87	Methyl salicylate	1781	A, B, C	16.29	1.14
88	Ethyl phenylacetate	1786	A, B	6.34	0.69
91	Ethyl salicylate	1816	A, B, C	8.98	0.99
97	Phenyl methyl pentanoate	1894	A	31.01	5.50
98	Benzyl isovalerate	1894	A, C	5.55	0.64
101	1-Butanol,3- methyl, benzoate	1916	A	53.76	8.04
107	2,6 Cresotic acid methyl ester (NIST)	1970	A	58.55	4.40
115	Methyl cinnamate	2078	A, B, C	0.55	0.13
118	Benzyl tiglate	2115	A	1.88	0.35
120	Ethyl cinnamate (NIST)	2135	A, B, D	21.22	1.25
122	Ethyl pentadecanoate	2148	A, C	2.64	0.24
124	Methyl isohexadecanoate	2166	A	5.38	0.45
126	Methyl hexadecanoate	2202	A, C, D	40.60	3.93
127	Butyl tetradecanoate	2216	A, C	22.65	2.44
128	Ethyl hexadecanoate	2251	A, C, D	239.63	11.34
130	Ethyl 9-hexadecenoate	2278	A	2.98	0.25
134	Methyl octadecanoate	2419	A, B, C	4.76	0.50
136	Methyl octadecenoate	2445	A, C	6.24	0.67
138	Ethyl octadecanoate	2458	A, C	12.61	0.76
139	Ethyl, 9-octadecenoate	2477	A	20.80	0.66
141	Ethyl 9,12-octadecadienoate	2527	A	13.11	0.79
143	Ethyl 9,12,15-octadecatrienoate	2594	A	12.38	1.14
144	Benzyl benzoate	2644	A, B	28.89	1.87

See footnotes to Table 1.

2.3. GC–MS Analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used. Injector temperature, 260 °C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 μm film thickness (Chrompack Italy s.r.l., Milan, Italy). The oven temperature was programmed as follows: 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C/min, and to 240 °C at 2 °C/min. The carrier gas was helium used at a constant pressure of 10 psi; the transfer line temperature, 250 °C; the ionisation mode, electron impact (EI); acquisition range, 40–200 m/z; scan rate, 1 μ s^{-1}. Each compound was identified using the NIST library (NIST/EPA/NIH Mass Spectra Library, version 1.7, USA), the linear retention indices (LRI) and authentic standard, where available. The linear retention index (LRI) was calculated according to Van den Dool and Kratz equation (1963).

Quantitative results were obtained using the method of internal standard: aliquots of an aqueous solution of 1-butanol (1 mg/ml) were added in the slurry prior to extraction. The coefficient of variation (CV%) for the three replicates of the same sample was inferior to 13.9, for all the analyzed compounds.

3. Results and discussion

The GC–MS chromatogram of the flavour profile of capers from the island of Salina (Eolian Archipelago) is shown in Fig. 1. In all, 145 different volatile compounds were identified and grouped in classes of substances (Tables 1–9). Cinnamaldehyde ($X = 396.63$ ppm) and benzaldehyde ($X = 311.34$ ppm) were the most abundant aldehydes; the volatile phenylpropanoid are important flavour constituents in herbs and spices and are involved in plant–animal interaction. For example, cinnamaldehyde commonly used in perfumes for its sweet and fruity scent is also used as fungicide typically applied to the root systems of plants; cinnamaldehyde is an effective insecticide and its scent is also known to repel animals like cats and dogs. Ethyl hexadecanoate ($X = 239.63$ ppm) was the major ester and, ethyl and butyl octanoate were also well represented. Free octanoic acid ($X = 730.05$ ppm) was the major constituent among the free acids, which, altogether, accounted for the 15% of the identified chemical constituents. Most fatty acids occur in nature as esters or are converted to alcohols, aldehydes, olefins, hydrocarbons and other secondary metabolites. Especially alcohols and aldehydes, derived from oxidative degradation of fatty acids, form the green leaf volatile complex of many plants. Terpenes constituted the 5.8% of the aroma; five sesquiterpenes (C-15) and ten monoterpenes (C-10) were identified for the first time in capers, with the acyclic sesquiterpene *trans*-nerolidol ($X = 137.2$ ppm) as the major one, followed by the monoterpene 4-terpineol ($X = 48.09$ ppm). Previously, Brevard et al. (1992) reported the monoterpene linalool and the sesquiterpene β-ionone as the only detected terpenes in capers from Morocco. The C-10 monoterpenes are often partially responsible for the odour of plants; their production often

Table 3
Aldehydes (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Aldehydes	LRI[b]	R[c]	X[d]	SD[e]
3	3-Methyl butanal	949	A	14.24	1.45
4	Pentanal	982	A, B, D	4.93	0.97
6	(*E*)-2-Butenal	1043	A, C, D	1.38	0.26
9	(*E*)-2-Pentenal	1133	A, B, C	1.34	0.28
13	Heptanal	1187	A, C	3.80	0.72
18	(*E*)-2-Hexenal	1222	A, B, C, D	5.10	0.91
25	Octanal	1291	A, B, C	0.34	0.08
28	(*E*)-2-Heptenal	1328	A, B, C	21.78	0.73
32	Nonanal	1396	A, B, C	11.32	0.69
34	(*E*,*E*)-2,4-Hexadienal	1407	A, B, C	7.48	0.54
42	Furfural	1467	A, C, D	29.88	1.24
45	(*E*,*E*)-2,4 Heptadienal	1497	A, B, C, D	52.92	3.03
48	Benzaldehyde	1529	A, B, C, D	311.34	16.16
50	(*E*)-2-Nonenal	1542	A, B, C, D	12.25	1.28
54	2,2-Dimethyl 3,4-pentadienal	1573	A	117.88	8.29
55	5-Methyl furancarboxaldehyde	1575	A	2.61	0.20
58	(*E*,*Z*)-2,6 Nonadienal	1588	A, B, C, D	5.32	0.37
66	5-Ethyl 2-furaldehyde	1641	A, B	6.63	0.45
67	(*E*)-2-Decenal	1645	A, C	7.93	0.64
77	(*E*,*E*)-2,4-Nonadienal	1704	A, B, C	7.41	1.34
78	4-Ethyl benzaldehyde	1714	A, B	26.68	2.23
82	2,4-Dimethyl benzaldehyde	1742	A, B, C, D	5.48	0.35
90	(*E*,*E*)-2,4-Decadienal	1810	A, B, C	10.83	1.33
113	(*E*)-Cinnamaldehyde	2044	A, B, C, D	396.63	26.08
131	Benzylacetaldehyde	2284	A	62.38	4.86
142	3-Methoxycinnmaldehyde	2565	A	27.78	1.74

See footnotes to Table 1.

Table 4
Ketones (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Ketones	LRI[b]	R[c]	X[d]	SD[e]
24	Acetoin	1289	A, B, C, D	3.10	0.17
29	6-Methyl 5-hepten-2-one	1338	A, B	3.06	0.18
47	(*E*,*E*) 3,5-Octadiene-2-one	1521	A, C, D	33.14	2.26
69	Acetophenone	1656	A, B, C	19.22	1.73
121	4′-Methoxy acetophenone	2144	A, B	1.97	0.19

See footnotes to Table 1.

Table 5
Alcohols (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Alcohols	LRI[b]	R[c]	X[d]	SD[e]
10	1-Butanolo	1136	A, B, C	82.77	0.03
15	2-Methyl 1-Butanol,	1201	A, B, C	1.66	0.33
30	3-Octanol	1386	A, B, D	2.34	0.21
38	1-Octen-3-olo	1442	A, B, C, D	15.77	0.75
44	2-Ethyl 1-hexanol	1483	A, B, C	7.80	0.38
52	1-Octanol	1552	A, B, C	11.68	1.47
56	4-Decanol	1581	A	0.93	0.22
92	*p*-Allylanisole	1828	A, B	5.70	0.54
96	Benzyl alcohol	1874	A, B, C, D	219.89	12.09
100	Phenyl ethyl alcohol	1909	A, B, C	40.16	1.86
110	4-(1-Methyl propyl) phenol	2012	A	109.23	7.34

See footnotes to Table 1.

Table 6
Sulphur compounds (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Sulphur compounds	LRI[b]	R[c]	X[d]	SD[e]
1	Carbon disulfide		A	9.42	0.93
2	Dimethyl sulfide	763	A, D	1.27	0.18
12	2-Ethyl thiophene,	1179	A, B, C, D	0.76	0.15
14	Isopropyl thiocyanate	1190	A	4.92	0.52
20	Methyl isothiocyanate	1243	A, B, C, D	441.22	29.22
106	Benzothiazole	1959	A, B	4.96	1.15
117	Benzyl isothiocyanate	2109	A, B	17.62	1.24

See footnotes to Table 1.

occurs in non-photosynthetic tissues in non-pigmented plastids known as leucoplast during the relatively brief periods when these cells are metabolically active (Gershenzon & Croteau, 1990). The analysis of volatile terpenes has been of considerable value for the resolution of systematic problems and many of them serve as chemical messages for insects and other animals. Moreover, their antimicrobial activity is linked to many human uses of monoterpenes, including medicinal and food uses (Beir & Nigg, 1992). Sulphur compounds accounted for the 8.42% of the volatiles, with methyl-isothiocyanate ($X = 441.22$ ppm) and benzyl-isothiocyanate ($X = 17.62$ ppm) as the major ones; both substances having a pungent smell and sharp test. This can be directly linked with the report by Matthaus and Ozcan (2002), on the high glucocapperin content in the buds of *C. spinosa* from Turkey, where glucocapperin accounted for the 80% of the total glucosinolates in those buds. In fact, isothiocyanates (ITCs) encountered in higher plants invariably derive from glucosinolates by enzymatic hydrolysis, which is, schematically shown in Fig. 2 for the conversion of glucocapperin into methyl-isothiocyanate. All *Capparis* species are sources of glucosinolates, and hence of isothiocyanates, with the structurally simplest representative, methyl glucosinolate (glucocapperin) as the apparently most widely distributed one. Recently, Zhang (2004) has documented the cancer-preventive activity of a significant number of isothiocyanates, the majority of which occur in plants, especially in cruciferous vegetables. Moreover, glucosinolates via their hydrolysis products are among the most powerful antibiotic substances known from higher plants (Louda & Mole, 1991), with an established correlation between the content of glucosinolates (isothiocyanates) and disease resistance (Chew, 1988). On the other hand, it is also known that (Mawson, Heaney, Zdunczyk, & Kozlowska, 1994) glucosinolates degradation products may adversely affect animal growth and repro-

Table 7
Hydrocarbons (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Hydrocarbons	LRI[b]	R[c]	X[d]	SD[e]
21	Styrene	1263	A, B	1.33	0.24
27	Tridecane	1299	A, B, C	3.78	0.77
33	Tetradecane	1400	A, B, C	27.97	4.06
36	Cyclotetradecane	1430	A	12.49	2.14
40	4-Methyl tetradecane	1455	A	19.26	2.22
46	Pentadecano	1501	A, B, C	139.18	14.92
51	Cyclopentadecane	1550	A	2.22	0.17
53	4 Methyl pentadecano	1555	A, C	18.40	2.03
60	Hexadecane	1599	A, B, C	15.74	1.53
68	4-Methyl hexadecane	1655	A	32.47	2.93
76	Heptadecane	1701	A, B, C	50.50	3.86
85	4-Methyl heptadecane	1754	A	25.37	2.26
89	Octadecane	1799	A, B, C	13.70	0.90
94	4-Methyl octadecane	1852	A	54.11	3.54
99	Nonadecane	1899	A, B, C	31.65	3.60
105	4-Methyl nonadecane	1953	A	5.92	1.08
109	Eicosane	1999	A, B, C	26.32	2.02
116	Heneicosane	2099	A, B, C	50.06	5.07
123	4-Methyl heneicosane	2152	A	22.67	1.55
125	Docosane	2201	A, B, C	18.64	1.75
132	Tricosane	2299	A, B, C, D	33.58	2.13
133	4-Methyl tricosane	2352	A	20.89	1.61
137	4-Methyl tetracosane	2453	A	66.65	4.23
140	Pentacosane	2499	A, B, C	22.89	1.11
145	4-Methyl hexacosane	2654	A	14.87	0.80

See footnotes to Table 1.

Table 8
Terpenes (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N[a]	Terpenes	LRI[b]	R[c]	X[d]	SD[e]
7	β-Pinene	1108	A, B, C	3.24	0.40
11	delta-3-Carene	1148	A, B, C	9.33	0.98
16	Limonene	1204	A, B, C	7.63	0.26
23	β-Cimene	1277	A	10.82	1.22
43	*p*-Menthone	1472	A, B, C	25.36	2.85
49	β-Linalool	1541	A, B, C, D	5.19	1.25
57	D-Fenchyl alcohol	1584	A, B, C	11.66	1.31
59	(−)Menthol	1596	A, B, C	4.61	0.52
61	4-Terpineol	1604	A, C	48.09	5.41
65	Isomenthol	1639	A, B, C	25.39	2.82
71	β-Farnesene	1667	A, C	5.93	1.07
74	Alfa-terpineol	1695	A, B, C	29.00	3.27
75	Methylgeranate	1696	A	3.53	0.66
79	(*E*,*Z*)-Alfa farnesene	1726	A, C	1.63	0.30
81	Carvone	1739	A, B, C	3.08	0.34
84	(*E*,*E*)-Alfa-farnesene	1749	A, C	4.37	0.77
86	Alfa-curcumene	1777	A, C	1.97	0.34
103	(*E*)-β-Ionone	1940	A, B, C, D	46.12	2.53
108	D-Nerolidol	1993	A, B, C	16.94	2.92
112	*trans*-Nerolidol	2035	A, B, C	137.20	23.80
119	Hexahydrofarnesyl acetate	2122	A	12.98	0.48

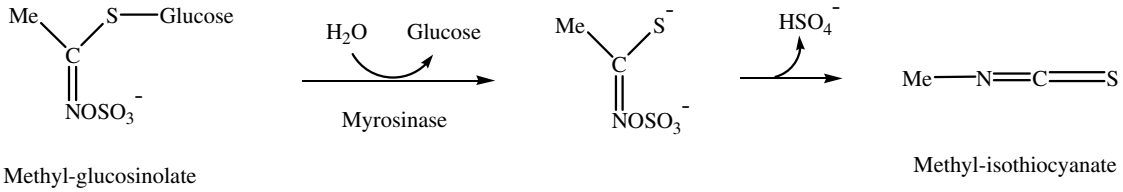
See footnotes to Table 1.

duction as well as palatability of fodder. In contrast to Brevard et al. (1992), we did not identify elemental sulphur (S_8) among the volatile constituents of Eolian capers; this might be due to our different extraction technique. Among other chemical classes, hydrocarbons (12.8%) and alcohols (7.48%) were also well represented in the aroma of the Eolian capers. In conclusion, the flavour profile of Eolian capers has been investigated for the first time, and the potential of the solvent-free HS-SPME extraction technique combined with the GC–MS analysis, as a possible simple routine method for analyzing food flavour, has been shown. Obviously, it should always be taken into consideration that the volatiles that form fruit flavour are produced through metabolic pathways during ripening, harvest,

Table 9
Miscellaneous compounds (ppm) in the volatile fraction of pickled flower buds from *Capparis spinosa* L.

N^a	Miscellaneous compounds	LRI[b]	R^c	X^d	SD[e]
73	*p*-Methoxystyrene	1680	A	20.43	1.48
83	Naphthalene	1749	A, B	3.16	0.30
95	2-Naphathalenon	1858	A	2.80	0.24
111	Diphenyl ether	2017	A, B	0.50	0.14

See footnotes to Table 1.



Methyl-glucosinolate

Methyl-isothiocyanate

Fig. 2. The myrosinase-catalyzed conversion of methyl-glucosinolate (glucocapperin) to methyl-isothiocyanate.

post-harvest and storage, and depend on many factors related to the species, variety and the type of technological treatment.

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