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VOLATILE AROMA CONSTITUENTS OF ORANGE

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Abstract–The aroma volatiles from the peel of oranges cultivated in Libya were analysed using routine procedures. Sixteen of the identified components have not previously been reported as orange volatiles, including sabinol, 4-methylacetophenone, hexyl hexanonate, γ-selinene and bisabolene. Whilst limonene was the major volatile component (*ca* 52% w/w of the total volatiles), this is a much lower concentration than is normally claimed for orange peel. However, other important aroma components were found at higher concentrations than usually reported, in particular, linalool (15.8%), geranial (3.5%), β-copaene (4.5%) and decanal (2.2%).

INTRODUCTION

There have been many studies of the volatile aroma components of oranges, and many volatiles have been identified and reported [1]. However, to date there has been no analysis of the aroma components of oranges cultivated in Libya, which are reputed to possess unusual, characteristic flavour. This paper reports the results of an investigation of the aroma volatiles of Libyan oranges.

RESULTS AND DISCUSSION

Because of the variation in the nature of the flavour components in different parts of the orange, most investigations of orange aroma volatiles have selectively examined only one specific part of the fruit, rather than the whole orange. Orange peel has been extensively studied, and peel oil is widely used as a flavour enhancer in fruit drinks, due to its unique and pleasant flavour [2]. Furthermore, most of the compounds which contribute to the characteristic aroma of orange juice originate in the peel [3]. For these main reasons, it was the peel of the Libyan oranges which was studied in this project.

Aroma extracts of the peel were prepared using well-established procedures, and were concentrated by high vacuum–low temperature distillation [4]. The resultant essence was found, on appropriate re-dilution, to possess a strong characteristic orange aroma.

The samples were analysed by GC and GC/MS (Table 1). A number of GC columns was used, but mainly fused silica capillary columns containing either bonded-phase BP1 (equivalent to OV 101) or BP20 (equivalent to PEG 20M). The retention data given in Table 1 were obtained using a 25 m fused silica column (BP1). Literature Kováts retention indices [5, 6] of most components (on OV 101) are also included in the Table, and confirm the general elution sequence. The qualitative data in Table 1 were obtained using both capillary columns; some components were more readily identified by GC/MS using one particular phase. Where positive identities are given, the mass spectra obtained on GC/MS agreed with those in the literature.

The quantitative data in Table 1 show that in total *ca* 54.6 μg of aroma components were obtained per gram of orange peel. This is a rather higher concentration than is generally reorted, e.g. [7], and levels of about 30 ppm are more common for other oranges. Overall, 71 components were detected as orange volatiles, of which 49 (comprising *ca* 98.7% w/w of the sample) were positively identified, with a further 13 (*ca* 1.0%) partially characterized. The 9 (*ca* 0.3%) unidentified components are not included in Table 1, and were present in the sample in such low amounts that either no mass spectrum could be recorded or the spectrum was too poor for interpretation. Of the fully identified components, 16 are reported as orange volatiles for the first time, and these are indicated in Table 1 by '+'. However, this includes a series of nine uninteresting, long straight chain alkanes, from C_{21} to C_{29}, inclusive.

Of the other new orange volatiles, bisabolene is a common and widespread aroma component. It has previously been detected in another citrus fruit, namely lime [8, 9]. Longifolene (only tentatively identified in this work) and γ-selinene are not such common volatiles, but they too have also been previously identified in a citrus fruit, in this case mandarin [10]. None of the other four new orange volatiles has previously been reported in citrus fruit, although hexyl hexanoate is another very common and widespread aroma component [1]. 4-Methylacetophenone is a little less common, and sabinol has been found in only a few plants (mainly fruits and spices) [1]. With regard to 4-isopropenyl-3-methylenecyclohexene, this has not been reported before as an aroma volatile [1]. Although the mass spectrum of the orange component matched the literature spectrum nearly perfectly, the structure of this hydrocarbon is unusual and the identification needs confirmation.

The most important aroma constituents of orange peel are terpenes, mainly monoterpenes, and certain aldehydes. The most abundant component by a large margin, and at a very high level for a single aroma component, is limonene. Previous workers have generally reported it at concentrations of between 83 and 97% of the total volatiles [11–17], although somewhat lower levels have also been found [7, 18, 19]. We found only 52%, although in absolute terms this represents about the same amount (*ca* 29μg/g) as reported in most other quantitative studies, due, of course, to the rather higher total concentration of volatiles obtained in this work.

The main reason for the somewhat lower percentage of limonene in the Libyan oranges is the relatively high concentration of linalool (*ca* 16%, 4.7 μg/g). Generally, levels of between 0.3 and 5.3% have previously been reported [7, 12, 13, 15–18, 20, 21], although Schreier *et al.* [19] found a similar amount to that obtained in this work and also a similar ratio of linalool to limonene (*ca* 1:3.5). Whilst the amount of linalool determined in Libyan oranges is quite high, it is the principal oxygenated terpene of orange peel, and it is considered to be one of the three most prominent constituents of good quality peel oil [22].

In most previous work, myrcene has usually been found to be the second most abundant volatile constituent of orange peel [11, 12, 14–17, 20], rather than linalool, as found in this investigation. However, myrcene was a reasonably abundant component of the volatiles from the Libyan oranges (*ca* 3.5%, 1.9 μg/g), and indeed was thus present at absolute levels about the same as, or somewhat in excess of, those previously reported [7, 11, 12, 14–20].

Whilst citral is mainly responsibe for the unique character of lemons, it is only a minor constituent in oranges. Usually, rather more geranial than neral has been determined in orange volatiles–on average, about double [14, 19, 23]. We also found geranial to be the major isomer, but to a much greater extent (*ca* 3.5% compared with *ca* 0.2%).

Another monoterpene carbonyl compound identified in reasonable concentration in this work was carvone (*ca* 2.5%, 1.4 μg/g). This is well known as an oxidation product of limonene [24]. The only other monoterpene detected in Libyan oranges and worthy of comment is α-terpineol. This should be at a low level in carefully produced orange oil, but it increases with temperature abuse of the oil [25]. The amount that we detected was low (*ca* 0.4%, 0.2 μg/g), and is of the same order as that reported by previous workers [7, 12, 13, 15, 17–20].

With regard to sesquiterpene hydrocarbons, valencene is one of the most important in oranges, and its very presence distinguishes the fruit from other citrus fruit [26]. However, it is not found in very high concentration, and the 0.4% (0.2 μg/g) determined in this investigation is in agreement with previously reported quantities [7, 15–19]. Valencene is present in the cuticular wax of oranges, and is believed to contribute to the persistent

Table 1. Volatile components of orange

Component	New	R_t (min)*	Kováts index (lit.)†	% rel. abund.	μg/g
α-Pinene		9.8	942	0.2	0.1
Sabinene		11.8	976	0.2	0.1
Myrcene		12.8	986	3.5	1.9
Octanal		13.1	985	0.2	0.1
α-Phellandrene		13.2	1002	0.4	0.2
Car-3-ene		13.6	1005	0.7	0.4
p-Cymene		14.4	1020	0.2	0.1
Limonene		15.0	1030	52.0	28.6
cis-β-Ocimene		15.6	1025	0.4	0.2
γ-Terpinene		16.0	1057	0.4	0.2
Terpinolene		17.4	1083	0.7	0.4
Octan-1-ol		17.5	1061	0.2	0.1
Nonanal		18.1	1087	2.4	1.3
Linalool		18.3	1092	15.8	8.7
4-Isopropenyl-3-methylenecyclohexene	+	19.0		0.5	0.3
Limonene-1,2-epoxide		19.3	1119	0.2	0.1
p-Mentha-2,8-dien-1-ol		19.6	1120	0.7	0.4
Sabinol	+	20.0	1130	0.2	0.1
Isopulegol		20.2	1133	0.4	0.2
4-Methylacetophenone	+	21.8	1166	0.4	0.2
α-Terpineol		21.9	1177	0.4	0.2
exo-4,7-Dimethylbicyclo[3.2.1]oct-3-en-6-one		22.0		0.4	0.2
Ethyl octanoate		22.5	1180	tr	tr
Decanal		22.6	1188	2.2	1.2
Carveol		23.2	1209	0.2	0.1
Monoterpene		23.3		0.2	0.1
Monoterpene		23.5		0.2	0.1
Neral		23.6	1220	0.2	0.1
Carvone		24.0	1223	2.5	1.4
Monoterpene		24.5		tr	tr

Table 1. (Continued)

Component	New	R_t (min)*	Kováts index (lit.)†	% rel. abund.	μg/g
Piperitone		24.8	1231	tr	tr
Geranial		26.0	1260	3.5	1.9
Terpene		26.5		0.2	0.1
Perillyl alcohol		27.5	1281	0.5	0.3
α-Cubebene		30.8	1360	0.2	0.1
Hexyl hexanoate	+	30.9	1371	0.2	0.1
β-Elemene		31.1	1400	0.4	0.2
β-Farnesene		32.7	1420	0.4	0.2
Sesquiterpene hydrocarbon		32.9		tr	tr
Caryophyllene		34.0	1440	0.2	0.1
Sesquiterpene hydrocarbon		34.4		tr	tr
? Longifolene	+	34.9	1450	0.2	0.1
γ-Selinene	+	35.2		0.2	0.1
Sesquiterpene hydrocarbon		35.3		tr	tr
β-Copaene		35.6	1460	4.5	2.5
Sesquiterpene hydrocarbon		35.9		tr	tr
δ-Cadinene		36.6	1520	0.4	0.2
Sesquiterpene hydrocarbon		36.7		0.2	0.1
Sesquiterpene hydrocarbon		37.9		tr	tr
Bisabolene	+	38.8	1500	0.2	0.1
Sesquiterpene hydrocarbon		40.9		tr	tr
Valencene		42.2	1515	0.4	0.2
Methyl ester		51.8		tr	tr
Eicosane	+	57.4	2100	0.2	0.1
Docosane	+	60.5	2200	0.2	0.1
Tricosane	+	63.5	2300	0.4	0.2
Tetracosane	+	66.0	2400	0.4	0.2
Pentacosane	+	68.8	2500	0.4	0.2
Hexacosane	+	71.2	2600	0.2	0.1
Heptacosane	+	73.6	2700	0.2	0.1
Octacosane	+	75.9	2800	tr	tr
Nonacosane	+	78.0	2900	tr	tr

*Order of elution from a BP1 GC column.
† Lit. [5,6].
tr = trace.

odour detected on the hands after holding the fruit [27]. Other sesquiterpene hydrocarbons which have been reported to contribute favourably to the characteristic flavour of oranges are β-elemene, farnesene, β-caryophyllene, δ-cadinene and humulene [26, 28]. All but the last were found in the Libyan oranges, but the most abundant sesquiterpene hydrocarbon by some margin was β-copaene (*ca* 4.5%, 2.5 μg/g). This has not often been previously detected as a volatile from orange peel.

As mentioned earlier, apart from terpenes, the most important aroma components of orange peel are certain aldehydes. Octanal and decanal are the two main aldehydes, and, together with linalool, they are reputed to be the most important volatile constituents of orange peel [22]. Reported concentrations for octanal range from 0.2 to 2.8% [7, 12–15, 17–20, 23, 29], and for decanal from 0.1 to 0.7% [7, 14–20, 23, 29]. Whilst the amount of octanal determined in Libyan oranges was at the bottom end of the range (*ca* 0.2%), an appreciable excess of decanal was found (*ca* 2.2%). Generally, it is the even-carbon number aldehydes which are mainly found in oranges, but we detected a relatively large amount of nonanal (*ca* 2.4%, 1.3 μg/g), far more than is usually quoted [7, 14, 15, 17–19, 29]. The total aldehyde concentration of an orange increases with maturity, and so the aldehyde content of orange oils is commonly used as a commercial index of quality [27].

A very interesting identification in this work was a novel bicyclic ketone, *exo*-4,7-dimethylbicyclo[3.2.1]oct-3-en-6-one (*ca* 0.4%, 0.2 μg/g). This, together with the *endo* isomer, was reported for the first time by Köpsel and Surburg [30], who identified the new naturally occurring compounds in Jaffa orange oil. They found that the *exo* isomer was the major constituent of the two, and it had a Kováts index of 1174 on a non-polar column similar to that used in this work. On synthesis, it was found to have a fresh camphoraceous and slightly sweet odour [30].

From the preceding summary, it is obvious that the aroma volatiles of this sample of the peel of oranges cultivated in Libya do indeed differ slightly, but significantly, from those normally found in other oranges. The differences are, however, mainly quantitative. In particular, the higher concentrations of the important aroma constituents, linalool, geranial, β-copaene and decanal, distinguish the Libyan fruit from others, and may well contribute to, and explain, the perceived difference in its flavour.

EXPERIMENTAL

Fresh, locally cultivated oranges were obtained from markets in Brack, Libya.

Sample preparation. Orange peel (530 g) was steam-distilled and the distillate extracted with CH_2Cl_2. The extract was then concd as previously described [4].

GC. FID-GC: 25 m × 0.2 mm i.d. fused silica capillary column coated with BP20 (or BP1) bonded phase; hydrogen, 1.2 ml/min; temp. programme, 70° for 5 min then 3°/min to 180°; detector and injection point heaters, 275 and 250°, respectively; injection volume, typically 0.1 μl at 25:1 split. A 5.5 m × 4 mm i.d. glass column packed with PEG 20M was also used.

GC/MS. A Kratos MS25 instrument was used, linked on-line to a Kratos DS55S data processing system. Capillary GC conditions as above were used, with He as carrier gas. The single-stage all-glass jet separator was at 250°. Significant operating parameters of the MS were: ionization voltage, 70 eV; ionization current, 100 μA; source temp., 225°; accelerating voltage, 1.33 kV; resolution, 1500; scan speed, 1 sec/decade (repetitive throughout run).

Quantitative assessment. Samples were prepared in such a manner that a known aliquot of the orange sample was analysed. Quantitative data were then derived both from the TIC monitor during GC/MS, and from the GC-FID trace during routine GC. EtOAc (0.050 M) was used as quantitative GC standard and corrections were made for the carbon-number of the identified constituents. An average correction factor was applied to unidentified GC peaks.

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