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Short Communication

A comparison of the peel oil components of Australian native lime (*Microcitrus australe*) and Mexican lime (*Citrus aurantifolia* Swingle)

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Abstract: The essential oil components extracted from the pericarp layer of two varieties of lime fruit, viz. Mexican lime (*Citrus aurantifolia* Swingle) and an Australian native lime (*Microcitrus australe*) have been analysed by gas chromatography/mass spectrometry. Thirty-three components were identified in *M australe* and 34 in *C aurantifolia*. The compound types comprised monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes and coumarins. For the more volatile monoterpenoid compounds, the major component was limonene, with significant amounts of γ-terpinene, β-pinene, geranial, neral, neryl acetate and geranyl acetate. From an examination of the nature and contents of individual components, there was no indication that any one compound might be responsible for the predominant aroma impact. The possible contribution to aroma differences due to quantitative differences in the amounts of these components is discussed. However, sensory evaluation indicated that there was little or no difference between the aromas of the two oils.

Keywords: aroma-impact components; Australian native lime; *Citrus aurantifolia* Swingle; essential oils; gas chromatography–mass spectrometry; GC-MS; Mexican lime; *Microcitrus australe*

INTRODUCTION

Australia has four varieties of native lime fruit that are found only in Australia. These were formerly classified as *Microcitrus* species, but they have recently been re-classified under the *Citrus* genus. The exact re-classification has not yet been published (V Cherikoff, personal communication) so, for this publication, we shall continue to use the *Microcitrus* classification for these Australian varieties. Of the four varieties, three are found in rainforest areas. These are *M australe* (commonly known as native lime), *M australiasica* (finger lime) and *M inodora* (North Queensland lime). The remaining variety is *M garrawayae*, which grows in the desert and is commonly known as desert lime.

Shaw *et al*[1] identified 53 components in the juice and 20 in the peel oil of *M inodora*. We have found no other paper that has reported on any aspect of Australian native limes.

In the present communication, we report on the volatile components of the oils that were solvent-extracted from the rinds of *M australe* and from the commonly available Mexican lime, *C aurantifolia* Swingle. We also comment on a number of the components that may contribute to lime aroma.

MATERIALS AND METHODS

Materials

Native lime fruits (*M australe*) were provided by Cherikoff Pty Ltd, Castle Hill, Sydney. Mexican Lime fruits (*C aurantifolia* Swingle) were grown and donated by Ms Thai-Ann Chorr.

Oil extraction

Using a sharp knife, the entire pericarp was cut from the fruit and sliced into narrow strips (*ca* 1 mm), and these were suspended in dichloromethane (50 ml) in a 150 ml Erlenmeyer flask. *N*-tridecane was added as internal standard both for quantitation of extracted oil and to determine when extraction had been completed. Aliquots of the supernatant liquid were sampled for GC analysis a number of times up to 48 h. For the analyses reported, extraction time was 48 h, although extraction was found to be complete after 24 h.

Gas chromatography–mass spectrometry

Oil analyses were effected by a Hewlett Packard (Avondale, VA, USA; gas chromatograph model

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5890, series II), to which was attached a quadrupole MSD (Hewlett Packard, Series 5972). The GC-MS system was controlled by a Hewlett Packard Chem Station Series G1034C, equipped with NBS-75K Library Search Software. The sample was introduced to the GC column in split mode by way of a high speed autoinjector (Hewlett Packard Model 6890; split ratio 35:1). The GC column used was a fused silica DB-5MS–5% phenyl–95% methylpolysiloxane bonded, 30 m in length, 0.25 mm id and 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). Injector and detector temperatures were set at 250 °C. Helium carrier gas pressure was 35 kPa at 60 °C and the flow rate was maintained constant at 32.0 cm s^{-1}. (0.94 ml min^{-1}) throughout the temperature programme by an electronic pressure programme (EPP). Initial column temperature was 60 °C, followed by a 3 °C min^{-1} programme to 180 °C. The MS filament was turned off for the first 3 min of analysis to allow time for the solvent to pass through the detector.

RESULTS AND DISCUSSION

Fruit characteristics

The fruit of Australian native lime (*M australe*) is approximately 20–30 mm in diameter. It has a round shape and the colour is light green to yellow. The Mexican lime (*C aurantifolia* Swingle) is the variety most widely cultivated in the world. The fruit is green when immature and pale yellow when mature, with a diameter of 2.5–5 cm.[2] Details of the extraction experiments are given in Table 1.

Table 1.

	M australis	*C aurantifolia*
Fruit weight (g)	21.00	45.42
Peel weight (g)	3.64	7.09
Peel (%)	17.39	15.62

Oil Isolation

Although a relatively slow technique, ambient temperature solvent extraction was selected as the preferred method, as the avoidance of any heat treatment reduced the possibility of degradation of labile compounds. Dichloromethane was selected as solvent because it has a low boiling point (40 °C), it can be readily freed by distillation from the small amounts of readily identifiable impurities that it commonly contains (mostly chloroform, benzene, toluene) and essential oils are highly soluble in it. In preliminary experiments (not reported), a number of variations of peel preparation were investigated, viz grating, and freezing and grating. All were more time-consuming and labour-intensive and none offered any advantage over the simple technique of suspending strips of peel in the solvent and swirling occasionally. It was determined that extraction of oil was complete within 24 h, with slight variability, presumably caused by variation in the degree of disruption of the oil cells, or the amount of mesocarp included with the pericarp slices during preparation of the rind for extraction. Analyses now reported were obtained by extraction at ambient temperature for 48 h. *M australe* was found to contain 0.22% and *C aurantifolia* 0.41% oil, calculated on the basis of the whole fruit. However, because of the paucity of sample available, these figures were obtained from analysis of only a single fruit of each variety. Therefore, the figures must be considered as indicative of oil content, rather than a statistically significant estimate.

Gas chromatographic parameters

The column selected (J&W DB5-MS) and the GC temperature programme were those reported by Adams.[3] However, because our GC was equipped

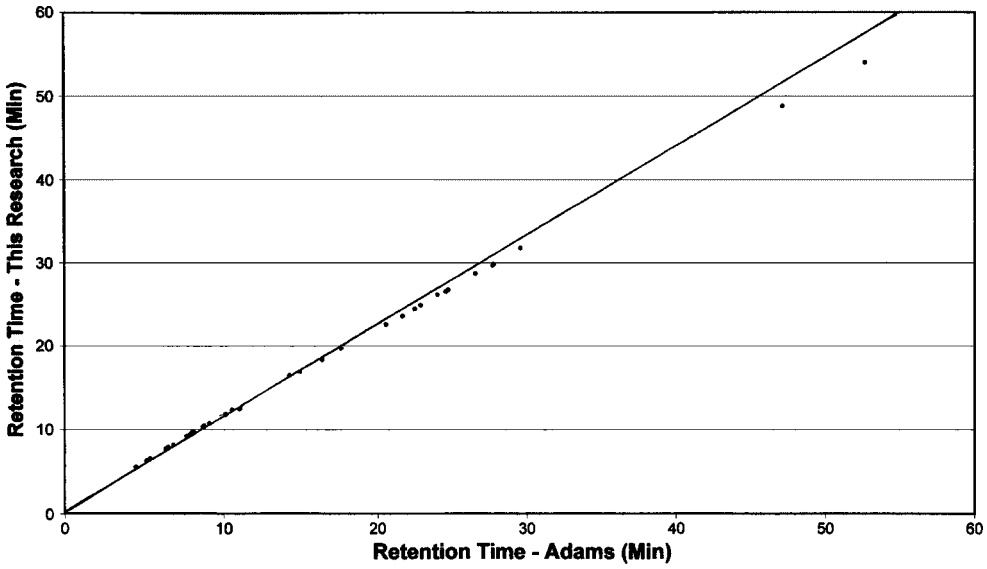

Figure 1. Comparison of retention times reported by Adams[3] and those of this research for *M australe*.

with an EPP control and that used by Adams was not, the flow rates profiles of our analyses were different from those reported by Adams. The impact of this difference is discussed below. The benefit of using this near-identical programme is also discussed below.

Mass spectral identification

Enzell *et al*[4] noted that terpenoids usually lack strong fragmentation-directing groups and that only in favourable cases may complete structural assignments be made by mass spectrometry alone. Adams (3) has promoted the concept of combining mass spectral information with GC retention times determined using a 5% phenylpolymethylsiloxane column and standardized GC temperature and carrier gas flow parameters. With the exception of the above-mentioned difference in flow rate profile, we have used the same experimental conditions in the present work. To better correlate our findings with those of Adams and to reduce the effects of minor differences in experimental technique, we plotted retention times of compounds that we can identify unequivocally with those reported by Adams. A linear correlation might be expected. In practice, we invariably obtained an excellent linear correlation only through the early part of the monoterpene range but, thereafter, the retention times quoted by Adams become progressively longer than those that we determined. The resultant relationship is thus slightly curvilinear for the longer retention time compounds that we have been able to identify unequivocally. It is apparent from GC parameters quoted by Adams that he used a GC that did not have EPP, so that his flow rate would have decreased slightly throughout the analysis, thereby giving increasingly higher retention times with increasing temperature, by comparison with analyses run with EPP control. In Fig 1, this early linear relationship and later progressive departure therefrom is illustrated. To progressively improve the probability of identification, we used a sequence of techniques to identify terpenoid components of the oils:

(1) individual spectra from beginning to end of each GC peak were examined to assess uniformity within the peak;
(2) the best quality spectrum from each peak was compared with that of the best match in the NBS75K library, account being taken also of the computer-determined quality match;
(3) retention times of well-identifiable components were plotted against those reported by Adams and the curvilinear relationship determined through the monoterpene and sesquiterpene regions; for the remaining components, we calculated the retention times that we would have expected Adams to have found; we then matched spectra that Adams reported for compounds at, or near, those retention times;
(4) retention times and spectra were matched against spectra and times of compounds that we have previously identified and collected in our own library;
(5) for sesquiterpene hydrocarbons, spectra and relative retention times were compared both with those of Adams and with those reported by Joulain and König;[5] These authors also used a 5% phenylpolymethylsiloxane column, but manufactured by Chrompack. However, relative retention times should be closely comparable.

Components identified are collected in Table 2.

Sensory evaluation

Sensory evaluation of the whole oils using a perfumer's strip indicated that there was little or no difference between the aromas of the two oils. The *C*

Table 2. Components Identified in lime oils

		M australe		*C aurantifolia*	
Peak no	Identity	R_t (minr)	Area (%)	R_t (minl)	Area (%)
1	α-Thujene[a,b,c]	6.3	0.2	6.3	0.4
2	α-Pinene[a,b,c,f]	6.5	1.3	6.5	1.4
3	Sabinene[a,b,c,f]	7.7	2.2	7.7	1.3
4	β-Pinene[a,b,c,f]	7.9	13.1	7.9	7.9
5	β-Myrcene[a,b,c,f]	8.2	1.0	8.2	1.0
6	α-Terpinene[a,b,c]	9.2	0.1	9.2	0.3
7	*p*-Cymene[a,b,c]	9.5	0.1	9.5	0.2
8	Limonene[a,b,c,f]	9.7	35.1	9.7	30.5
9	1,8-Cineole[a,b,c]	ND[(e)]		9.8	0.4
10	β-Ocimene (*E*-)[a,b,c,f]	10.3	0.2	10.3	0.2
11	γ-Terpinene[a,b,c]	10.8	11.2	10.8	19.2
12	Terpinolene[a,b,c,f]	11.8	0.4	11.8	0.8
13	Linalool[a,b,c,f]	12.4	0.3	12.4	0.2
14	α-Terpineol[a,b,c,f]	16.6	0.7	16.5	0.7
15	Decanal[a,b,c,f]	17.0	0.8	17.0	0.2
16	Neral[a,b,c,e,f]	18.4	4.5	18.4	3.8
17	Geranial[a,b,c]	19.7	7.3	19.7	5.9
18	δ-Elemene[a,b,d]	22.6	1.2	22.6	0.5
19	Neryl acetate[a,b,c]	23.6	0.1	23.6	2.2
20	Geranyl acetate[a,b,c]	24.5	0.4	24.4	0.5
21	β-Elemene[a,b,c,d]	24.9	0.7	24.9	0.3
22	Unidentified	25.8	0.3	25.8	0.1
23	β-Caryophyllene (*E*-)[a,b,c,d]	26.2	1.0	26.1	0.7
24	γ-Elemene[a,b,d]	26.6	0.2	26.5	0.1
25	α-Bergamotene[a,b,c,d]	26.8	1.6	26.7	1.7
26	Germacrene D[a,b,d]	28.7	0.5	28.6	0.2
27	α-Bisabolene (*Z*-)[a,b,d]	29.5	0.1	29.5	0.2
28	α-Farnesene (*E,E*-)[a,b,c,d]	29.7	3.3	29.6	1.4
29	β-Bisabolene[a,b,c,d]	29.8	2.8	29.8	2.8
30	Germacrene B[a,b,d]	31.8	0.9	31.7	0.3
31	7-methoxycoumarin[a]	37.9	1.1	37.9	3.3
32	5,7-Dimethoxycoumarin[a]	46.5	3.4	46.5	6.6
33	*Iso*-bergaptene[a,b]	48.1	0.2	48.0	0.4
34	Bergaptene[a,b]	48.8	0.4	48.8	2.9
35	Isopimpinellin[a,b]	53.9	3.5	53.8	1.8

R_t, retention time. [a] Spectrum matches NBS75K library spectrum. [b] Spectrum matches Adams[3] and retention time correlates. [c] Spectrum and retention time matches own files. [d] Spectrum matches Joulain and König.[5] [e] Not detected. [f] Also identified in *M inodora* by Shaw *et al*.[1]

aurantifolia contained a small amount of 1,8-cineole but, otherwise, the two oils were qualitatively closely similar. There would thus appear to be no individual component that might contribute to differences in overall aroma quality.

In general, aroma-impact tends to increase for compounds of higher volatility and polarity. In the present case, this would include the monoterpene hydrocarbons and oxygenated monoterpenes, with possibly a minor contribution from some of the more volatile sesquiterpene hydrocarbons, such as β-caryophyllene (woody, spicy). For the more volatile monoterpenoid compounds, the major component was limonene, with significant amounts of γ-terpinene, β-pinene, geranial, neral, neryl acetate and geranyl acetate. All of these compounds have aroma impact, so differences in amount might be expected to contribute to overall aroma quality. As limonene has only a mild sweet, citrus aroma, the small difference between the amounts in the two oils should not be significant. Likewise, the combined contents of neral and geranial (*M australe*, 11.8; *C aurantifolia*, 9.7%), which both have strong and similar lemon aromas, are sufficiently close that one would not expect an easily detectable aroma difference. There was considerably more β-pinene in *M australe* than in *C aurantifolia* and, as β-pinene has a woody, pine-like aroma, this difference might be expected to be sensorially detectable. A difference in herbaceous aroma might be introduced to *C aurantifolia* because of its higher level of γ-terpinene. While all of the components that eluted up to β-caryophyllene would make some contribution to the overall aroma, it is not possible to predict their likely effect empirically. However, it is clear that the differences in composition are unlikely to be sufficient to give rise to significant aroma differences.

The later-eluting components can be divided into sesquiterpenoids and a number of coumarin derivatives. When one considers the lower aroma-impact of the sesquiterpene hydrocarbons, there are no differences in quantitative amount that would be expected to contribute significantly to aroma differences between the two oils. It is likely that the coumarins could contribute to the taste of these oils, but this aspect has not been investigated in this project.

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