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Effect of oxidative deterioration on flavour and aroma components of lemon oil

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ABSTRACT

Reactions of five major components (citral, α- and β-pinene, limonene and γ-terpinene) of lemon oil in the presence of Cu catalysts and air have been shown to lead to significant oxidation of α- and β-pinene and γ-terpinene, even when the catalyst concentration was comparable to that present in copper-plumbed tap water. Addition of commercial antioxidants (BHA and tocopherol) generally led to suppression of oxidation. UV degradation of these compounds in the presence of air was most significant for γ-terpinene and limonene which gave products similar to those obtained from the Cu-catalysed thermal reactions. Citral gave different products, mainly photocitrals, in contrast to the thermal reactions. The sensitivity of lemon oil to temperature and the presence of air was confirmed.

1. Introduction

Lemon oil and lemon essence are among the most useful flavouring materials for food, such as soft drinks, dairy products, candies and cakes. The high level of unsaturated and oxygen-functionalised terpenes in lemon oil render it very susceptible to oxidation. The resulting changes in peel oil constituents have long been known to be responsible for flavour changes in citrus juice during storage (Nagy & Rouseff, 1980). Oxidation has been shown to be influenced by temperature, UV irradiation and traces of metals as catalysts (Sinki, Assaf, & Lombardo, 1997). Citral is the major contributor to the flavour and aroma of lemon oil and lemon essence and other major constituents include D-limonene, γ-terpinene and α- and β-pinenes (Dugo, Cotroneo, Verzera, & Bonaccorsi, 2002).

The formation of *p*-cymene, which is a major contributor to off-flavours, can arise from oxidation of limonene and γ-terpinene, as well as from acid-catalysed cyclisation and dehydration of citral (Sinki et al., 1997). Many detailed studies of the composition of citrus oils have been carried out and recent work has focussed on the use of sensory chemical analysis to find the flavour components which have the highest sensory impact (Chida, Yamashita, Izumiya, Watanabe, & Tamura, 2006; Plotto, Margaria, Goodner, Goodrich, & Baldwin, 2004).

In this paper the susceptibility of five major components of lemon oil to oxidation in the presence of a copper oxide catalyst or under UV irradiation has been assessed in both the presence and absence of antioxidants. The reactions were carried out under conditions designed to accelerate the generation of off-flavours and facilitate the identification of degradation products. Changes in composition of the individual components have been assessed by GC–MS and changes in odour reported.

In addition, the effects of storage temperature, availability of oxygen and light on the degradation of samples of lemon oil itself were studied.

2. Materials and methods

2.1. Reagents and chemicals

Australian cold-pressed lemon oil (used within three months from date of manufacture) and individual lemon oil components, such as D-limonene, α-pinene, β-pinene, γ-terpinene and citrals (natural and synthetic) were supplied by BBA (Bush Boake Allen Pty-Ltd., Melbourne Australia). Citral, for the UV irradiation experiment, was obtained from Symrise AP Pty Ltd., Singapore. Butylated hydroxyanisole (BHA) and mixed tocopherols (vitamin E;

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DL-α-tocopherol purchased from BASF) were obtained from BBA (Bush Boake Allen).

2.2. Gas chromatography and mass spectrometry (GC–MS) experimental methods

All samples were analysed using gas chromatography–mass spectrometry (GC–MS). Electron impact (EI) mass spectrometric data were collected using a Hewlett–Packard 5973 mass spectrometer interfaced to a Hewlett–Packard 6890 gas chromatograph.

The sample was injected by using a Hewlett–Packard auto-sampler 7673. The injection volume was 0.1 μl. Separation of each compound was achieved on a fused silica capillary column (SGE, BP20), 60 m × 0.25 mm i.d., 0.25 μm film thickness. The injection temperature was 230 °C. The oven temperature programme was as follows: 50 °C at start, held for 5 min, then increased at 3.5 °C/min to 230 °C and increased at 5 °C/min until the final oven temperature was 235 °C and held for 13 min. The total run time was 70 min. Helium was used as the carrier gas with a split ratio of 25:1 and the split flow rate at 25 ml/min.

The mass spectrometric detector was set at 250 °C. Before analysis, *tert*-butyl alcohol was added to each sample as an internal standard (1% w/w). The samples (auto-sampler, 0.1 μl) were then analysed by GC–MS to obtain the composition of the oil and to detect the presence of oxidised compounds.

The amount of each compound was calculated by computing the area of each compound against the area of the internal standard in the gas chromatogram. The reproducibility of peak areas in the chromatograms was ±0.1%. Small amounts of compounds detected are listed as <0.1% and are not included in the calculation of the total (100%) response area for the GC reports. Determination of the relative GC response factors for the various lemon oil components requires a pure sample of each compound and was not possible because of the large number of lemon oil constituents and thus we report values as "relative response area percent".

The mass spectrum of each identified compound was compared to the actual retention time and the mass spectrum of each compound stored in the relevant standard databases (Wiley 275 and BBA mass spectral terpene databases).

2.3. Sensory evaluation

The general procedure followed was the standard industry procedure for evaluating lemon oil aroma. Sensory evaluation of all samples was achieved at room temperature under clean air conditions. The samples were taken out from the experimental storage conditions (namely 4, 30 or 50 °C) and allowed to stand at room temperature for 30 min (i.e. allowed to cool down or warm up, depending on the experimental storage conditions). Smelling strips were used to detect the odour. One end of a smelling strip (about 1 cm) was dipped into the sample. Three deep, quick sniffs were achieved from the smelling strip and then the odour source (i.e. smelling strip) was removed. Clean air was breathed between each assessment. A gap of 20 s was sufficient between individual odour assessments (Carpenter, Lyon, & Hasdell, 2000).

2.4. Experiments with lemon oil

2.4.1. Effect of storage at 30 °C in the dark and in light under N_2

Lemon oil samples were prepared as follows: six 15 ml clear glass bottles were filled up to the neck with lemon oil (*ca* 10 g each), flushed with pure nitrogen and closed with a screw-cap. The lemon oil samples were divided into two groups. The three bottles in the first group were covered completely with aluminium foil, put into a cardboard box and stored at 30 °C in an oven. The three bottles in the second group were left uncovered and stored at 30 °C in an oven near the clear glass door so the visible light could penetrate the samples. After four days, all the bottles were taken out for sensory evaluation of the aroma and GC–MS analysis. Each sample was analysed by GC–MS and the results of each set of three samples averaged (results ±0.1%). The samples stored in the dark were re-evaluated after two months.

2.4.2. Effect of storage temperature

2.4.2.1. Effect of increased storage temperature (50 °C). Three samples of lemon oil (8 g each) were weighed into 10 ml brown glass bottles, purged with pure nitrogen, closed with screw-caps and stored at 50 °C in an incubator oven. The samples were checked for aroma deterioration after four days and two weeks and analysed using GC–MS.

2.4.2.2. Effect of decreased storage temperature (4 °C). Four samples of lemon oil (8 g each) were weighed into 10 ml brown glass bottles, purged with pure nitrogen, closed with screw-caps and stored at 4 °C in the refrigerator. The samples were checked for aroma deterioration after 2 months, 6 months and 12 months and analysed using GC–MS.

2.4.3. Effect of headspace

2.4.3.1. Storage at 50 °C. Two sets of lemon oil samples were prepared. The first set of five samples (8 g each) was prepared in 10 ml brown glass bottles, the sample was filled up to the neck, leaving 1 cm headspace, and capped. The second set of five samples was prepared in the same manner but the bottles were only half-filled (using 4 g of lemon oil each). The bottles were stored at 50 °C in an incubator oven and the oil samples were evaluated after two weeks for aroma deterioration and analysed using GC–MS.

2.4.3.2. Storage at 4 °C. Two sets of five lemon oil samples were prepared as described above and stored in the refrigerator at 4 °C. The oil samples were evaluated after 12 months for aroma deterioration and analysed using GC–MS.

2.5. Catalytic oxidation of individual main lemon oil components

Catalytic oxidation of the main components present in lemon oil was carried out as exemplified by the description below for D-limonene. Two samples each, of D-limonene, citral, α- and β-pinene and γ-terpinene were treated in this manner. GC–MS results from such duplicate samples are average values (±0.1%). D-Limonene (10 g) and copper oxide (0.01 g) (0.1% CuO) were weighed into a glass bottle (50 ml) which was flushed with oxygen and sealed using a screw-cap. The sample was stirred at 40 °C for 4 h. Sensory-evaluation of the oxidised sample was carried out and compared against the control (fresh D-limonene) for deterioration of aroma. Both samples were then analysed using GC–MS (after addition of internal standard).

2.6. Effect of synthetic (butylated hydroxyanisole, BHA) and natural (mixed tocopherols, vitamin E) antioxidants on the rate of oxidation of the individual main components of lemon oil

2.6.1. With no antioxidants- as in Section 2.5 but with 0.01% CuO

D-Limonene, citral (neral and geranial), γ-terpinene, α- and β-pinene were used in this and the following experiment. For example, D-limonene (10 g) was weighed into a 50 ml glass bottle; copper oxide (0.001 g) (CuO, 0.01%) was added to the sample which was stirred using a magnetic stirrer for 5 min at ambient temperature (*ca* 25 °C). The bottle was flushed with pure oxygen, screw-capped and stored at 40 °C in a GC (gas chromatography) oven for 4 h. The samples were removed from the GC oven and left to cool to room temperature for 30 min (see Section 2.3). Aroma

evaluation of each sample against the fresh samples was carried out before the sample was injected for GC–MS analysis.

2.6.2. With antioxidants (BHA or tocopherols)

As an example, antioxidant (0.1%) was added to D-limonene (10 g) in a 50 ml glass bottle. The sample was stirred gently to dissolve the antioxidant and CuO (0.001 g, 0.01%) was added to the mixture and stirred for 5 min at ambient temperature. The sample was flushed with pure oxygen, screw-capped and kept at 40 °C in a GC oven for 4 h. The samples were analysed as described above.

2.7. Effect of ultraviolet (UV) light on the individual main components of lemon oil

Citral, D-limonene, γ-terpinene, α- and β-pinene were used in the experiment. The preparation of each sample was as follows: the sample (3 g) was weighed into a 25 ml Erlenmeyer flask, flushed with pure oxygen and the flask was sealed. The sealed flasks were irradiated for 48 h at 37 °C in a self-constructed light box with a UV lamp (40 W, range of UV light, 330–400 nm, Osram). After irradiation, the samples were removed for sensory evaluation against the fresh samples (stored at 4 °C). Hexane (1 ml) containing 1% of *tert*-butyl alcohol was added to dissolve each sample and the solutions analysed by GC–MS (GC–MS results for citral are average values for the three samples ±0.1%).

3. Results and discussion

3.1. Deterioration of lemon oil

The degradation characteristics of lemon oil samples under standard conditions were assessed and it was found that our samples were consistent with reported trends. Triplicate samples of cold-pressed lemon oil were kept in the dark at 30 °C under nitrogen and analysed by GC–MS after four days and two months. Similarly, an identical triplicate set of samples was stored at 30 °C and exposed to visible light through the glass door of the oven. The compositions of the fresh oil and the stored samples are given in Table 1. Results were reproducible to ±0.1%, and only changes $\geqslant$0.2% were considered relevant. Samples stored in the dark for two months showed significant losses of citral, α-terpinolene and α- and γ-terpinene, compounds considered to be major contributors to fresh lemon flavour (Schieberle & Grosch, 1989).

In addition, the amount of D-limonene was significantly decreased, which is probably one of the contributing factors leading to the increase in *p*-cymene (McGraw, Hemingway, Ingram, Canady, & McGraw, 1999), along with that generated from γ-terpinene (Freeburg, Mistry, & Reineccius, 1994). A decrease in the amount of α- and β-pinene was also noted after two months.

The effect of incident light was small, as after four days the compositions of the light and dark samples were very similar. However, the small differences in composition, e.g. in the slightly greater loss of limonene and citral, led to an observable difference in odour. The sample stored for two months in the dark also showed an off-lemon odour. The gassy, kerosene-like odour of *p*-cymene (Arctander, 1969) was not detected, possibly since it was depressed by the odour of other oxygenated components formed during oxidation, such as α-terpineol, epoxy-terpinolene, carveols and limonene oxides. Since the odour threshold of *p*-cymene is high, its contribution to the off-flavour of deteriorated lemon oil is not so significant compared to the effect of other terpene degradation products (Freeburg et al., 1994; Schieberle & Grosch, 1988).

Accelerated deterioration was studied over two weeks at 50 °C for samples under N_2, in fully-filled bottles (*ca* 1 cm headspace) and in half-filled bottles containing air (Table 2). Not surprisingly the samples stored under N_2 and in a filled bottle containing a minimum amount of air showed very similar changes. These changes were very similar to those shown in Table 1 for samples stored in the dark at the lower temperature (30 °C) but for longer time (two months). The samples in the half-filled bottles containing air, as expected (Iwanami, Tateba, Kodama, & Kishino, 1997; Nagy & Rouseff, 1980), showed significantly larger changes with large losses of limonene, γ- terpinene and citral, resulting in corresponding increases in *p*-cymene and geranic acid.

Table 1
GC–MS analysis of cold-pressed lemon oil stored at 30 °C under N_2 in the dark and in light

Relative response area (%)				
Compound	Fresh	Dark, four days	Light, four days	Dark, two months
α-Pinene	5.3	5.0	5.0	4.8
α-Thujene	0.3	0.3	0.3	0.2
Camphene	0.3	0.3	0.3	0.3
β-Pinene	15.0	14.9	14.9	14.3
Sabinene	1.7	1.7	1.7	1.5
δ-3-Carene	0.2	0.2	0.2	0.1
β-Myrcene	4.0	4.0	4.0	3.7
α-Terpinene	0.7	0.7	0.7	<0.1
D-Limonene	45.0	44.6	44.0	39.1
β-Phellandrene	0.6	0.6	0.6	0.5
(*Z*)-β-Ocimene	0.2	0.2	0.2	0.2
γ-Terpinene	15.6	15.6	15.5	11.7
(*E*)-β-Ocimene	0.4	0.4	0.4	0.4
p-Cymene	0.7	1.4	1.8	12.8
α-Terpinolene	1.3	1.3	1.2	0.8
n-Octanal	0.4	0.4	0.4	0.2
n-Nonanal	0.2	0.2	0.2	<0.1
(*Z*)-Limonene oxide	<0.1	0.1	0.1	0.8
(*E*)-Limonene oxide	<0.1	0.1	0.1	0.6
Epoxy-terpinolene	<0.1	<0.1	<0.1	0.2
Citronellal	0.3	0.3	0.3	0.3
Linalool	0.2	0.2	0.2	0.2
α-Bergamotene	0.3	0.3	0.3	0.3
β-Caryophyllene	0.8	0.8	0.8	0.5
(*Z*)-Citral	1.9	1.6	1.6	0.6
α-Terpineol	0.4	0.4	0.4	1.5
Neryl acetate	0.9	0.9	0.9	1.0
β-Bisabolene	—	0.4	0.5	0.4
(*E*)-Citral	2.3	2.0	1.9	0.9
Geranyl acetate	0.8	0.9	0.9	0.9
(*trans*)-Carveol	<0.1	<0.1	0.1	0.2
(*cis*)-Carveol	<0.1	<0.1	0.1	0.1
Caryophyllene oxide	—	<0.1	<0.1	0.2
Geranic acid	—	0.2	<0.1	0.9
Odour	Fresh lemon	Freshness reduced	Off-lemon odour	Off-lemon odour

'—' indicates below detection level; <0.1% indicates small amounts were detected; small amounts ($\leqslant$0.1%) of dihydrocarveol, geraniol, *n*-octanol, γ-terpineol, nerol, octanoic acid and nonanoic acid also detected.

3.2. Oxidation of individual components of lemon oil

Five of the major components of lemon oil, which were shown to be susceptible to deterioration in Section 3.1, were chosen for degradative studies under accelerated conditions. Samples were stored under oxygen in the presence of small amounts of copper oxide at 40 °C for 4 h or exposed to UV light for 48 h. Copper oxide was chosen as an example of a copper species which could be found on copper tubing and it has long been known to promote hydroperoxide formation (Burger, Meyer, Clement, & Balaceanu, 1961; Hock & Kropf, 1959). The effect of adding two commercial antioxidants was studied.

Table 2
GC–MS analysis of the effect of headspace on the storage of cold-pressed lemon oil at 50 °C

Relative response area (%)				
Compound	Fresh	Under N_2	Filled bottle[a]	Half filled bottle
α-Pinene	5.3	5.0	5.0	5.0
Camphene	0.2	0.1	0.1	<0.1
β-Pinene	14.7	14.6	14.6	14.2
Sabinene	1.8	1.6	1.6	1.4
δ-3-Carene	0.2	0.2	0.2	0.2
β-Myrcene	3.7	3.6	3.7	3.4
α-Terpinene	0.7	0.2	0.2	—
D-Limonene	45.8	40.1	40.1	36.5
β-Phellandrene	0.6	0.3	0.3	0.3
γ-Terpinene	15.4	13.5	13.4	10.3
(*E*)-β-Ocimene	0.2	0.1	0.2	0.1
p-Cymene	1.1	10.5	10.2	16.2
α-Terpinolene	1.2	0.9	0.9	0.7
n-Octanal	0.4	0.3	0.3	0.2
n-Nonanal	0.2	0.2	0.2	0.1
(*Z*)-Limonene oxide	<0.1	0.8	0.8	1.0
(*E*)-Limonene oxide	<0.1	0.4	0.4	0.5
(*Z*)-Sabinene hydrate	<0.1	<0.1	0.1	<0.1
Epoxy-terpinolene	—	0.2	0.2	0.2
Citronellal	0.2	0.1	0.1	0.1
Linalool	0.1	0.1	0.1	0.1
n-Octanol	<0.1	0.1	0.1	0.1
(*E*)-α-Bergamotene	0.2	0.3	0.3	0.3
β-Caryophyllene	0.9	0.4	0.4	0.4
Terpinene-4-ol	0.1	0.2	0.2	0.2
(*Z*)-Citral	2.0	0.4	0.4	0.2
α-Terpineol	0.4	0.8	0.8	0.5
γ-Terpineol	<0.1	<0.1	0.1	<0.1
Neryl acetate	0.9	0.9	0.9	0.9
β-Bisabolene	0.3	0.1	0.4	0.3
(*E*)-Citral	2.6	1.0	1.1	0.3
Geranyl acetate	0.8	0.8	0.8	1.1
Dihydrocarveol	—	0.1	0.1	<0.1
Nerol	<0.1	0.1	0.1	0.1
(*trans*)-Carveol	—	0.1	0.2	1.0
Geraniol	<0.1	0.1	0.1	0.1
(*cis*)-Carveol	—	0.1	0.1	0.1
Caryophyllene oxide	—	0.2	0.2	0.3
Octanoic acid	—	0.3	<0.1	0.1
Nonanoic acid	—	<0.1	<0.1	0.1
Geranic acid	—	1.2	1.1	2.6
Odour	Fresh lemon	Off-lemon odour, minty, herbaceous	Off-lemon odour	Off-lemon odour, slightly soapy

'—' indicates below detection level; <0.1% indicates small amounts were detected.
[a] 1 cm headspace.

3.3. Oxidation of citral

Storage of citral at 40 °C for 4 h under oxygen in the presence of CuO (0.01%) resulted in isomerisation of (*Z*)- to (*E*)-isomers and an overall decrease in citrals (0.7%) with a concomitant increase in geranic and neric acids (Table 3). A very similar result was obtained when the CuO level was increased to 0.1%. Addition of tocopherols suppressed isomerisation of (*Z*)-citral and limited the oxidation of both isomers. BHA also suppressed the oxidation of the citrals but did not prevent (*Z*)–(*E*) isomerisation.

UV irradiation of citral at 37 °C under oxygen for 48 h led to some (*E*) to (*Z*) isomerisation (*ca* 6%) with a small amount of photocitrals, predominantly photocitral A (0.6%) being formed in agreement with literature reports (Barany, Wolff, & Agosta, 1978; Cookson, Hudec, Knight, & Whitear, 1963; Wolff, Barany, & Agosta, 1980). The fresh lemon odour was reduced and a slightly dusty and earthy note developed. Iwanami noted similar odour changes on irradiation of citral in ethanol solution and of lemon essence and lemon drink (Iwanami et al., 1997).

Table 3
GC–MS analysis of the effect of antioxidants (BHA and tocopherols) on the oxidation of citral (O_2/CuO/40 °C/4 h)

Compound	Fresh	Relative response area%		
		CuO, 0.01%		
		No antioxidant	BHA	Tocopherol
(*Z*)-Citral	46.5	45.1	45.0	46.2
(*E*)-Citral	52.2	52.9	53.5	52.3
Neric acid	0.2	0.4	0.2	0.2
Geranic acid	1.1	1.6	1.3	1.3

3.4. Oxidation of β-pinene

Samples of β-pinene showed similar degrees of degradation in an oxygen atmosphere at 40 °C with CuO (0.1 or 0.01%) as catalyst or with UV irradiation (Table 4).

The same four products were formed and the odour changed from fresh pine to a slightly minty, turpentine-like off odour. These products, arising from allylic oxidation, have been reported previously (McGraw et al., 1999; Usai, Arras, & Fronteddu, 1992). Addition of BHA or tocopherols to reactions involving CuO (0.01%) led to a slight reduction in the loss of β-pinene.

3.5. Oxidation of D-limonene

Copper-catalysed oxidation (CuO, 0.1%) resulted in a low conversion of limonene to a mixture of limonene oxides, carvone and carveols, resulting in a change in odour with the development of piney, flowery notes (Table 5). A similar loss of limonene was observed using 0.01% CuO and this was prevented by the addition of either BHA or tocopherols.

In contrast, UV irradiation caused a substantial conversion of limonene to the same mixture of products, resulting in a caraway-like, minty odour (Table 5). The products formed were similar to those reported by others under a wide range of conditions (Kutty, Braddock, & Sadler, 1994; Soottitantawat et al., 2004).

The greater reactivity relative to β-pinene could be associated with the greater number of allylic hydrogens in D-limonene (11) versus β-pinene (3) giving greater opportunity to form resonance-stabilised allylic radicals by hydrogen atom abstraction.

Table 4
GC–MS analysis of β-pinene (CuO, 40 °C, O_2, 4 h or UV light, 37 °C, O_2, 48 h)

Compound	Fresh	Change in relative response area (%)				
		CuO, 0.1%	CuO, 0.01%			UV
			No antioxidant	BHA	Tocopherol	
β-Pinene	93.7	−4.5	−5.3	−3.7	−3.8	−5.9
α-Pinene	6.0	−1.2	−1.2	−0.6	−0.5	*
β-Myrcene	0.3	−0.3	−0.2	−0.2	−0.2	−0.3
Pinocarvone	—	+1.9	+2.0	+1.9	+1.9	+1.8
Pinocarveol	—	+1.5	+3.5	+2.0	+2.0	+2.3
Myrtenol	—	+1.4	+0.6	+0.3	+0.3	+1.2
Myrtenal	—	+1.2	+0.6	+0.3	+0.3	+0.9
Odour	Fresh, pine-like	Off odour, slightly minty, turpentine-like				

'—' indicates below detection level; * no α-pinene in this sample.

Table 5
GC–MS analysis of D-limonene (CuO, 40 °C, O_2, 4 h or UV light, 37 °C, O_2, 48 h)

Compound	Fresh	Change in relative response area (%)	
		CuO, 0.1%	UV
α-Pinene	1.7	−0.4	−0.4
β-Myrcene	6.8	−1.1	−1.1
D-Limonene	84.8	−1.0	−8.8
β-Phellandrene	0.9	−0.3	−0.3
p-Cymene	0.1	+0.1	nil
(*Z*)-Limonene oxide	0.2	+0.9	+2.3
(*E*)-Limonene oxide	0.1	+0.4	+2.1
Carvone	0.2	+0.5	+5.0
(*trans*)-Carveol	0.1	+0.5	+1.0
(*cis*)-Carveol	<0.1	+0.3	+1.9
Odour	Weak citrus-like	Very weak citrus, piney, flowery, minty.	Caraway-like, minty

Table 6
GC–MS analysis of α-pinene (CuO, 40 °C, O_2, 4 h or UV light, 37 °C, O_2, 48 h)

Compound	Fresh	Change in relative response area (%)	
		CuO, 0.1%	UV
α-Pinene	94.1	−17.5	−16.2
β-Pinene	2.0	−0.5	*
α-Pinene oxide	0.3	+7.4	+3.7
Verbenone	0.8	+1.4	+1.1
Verbenol	—	+4.8	+3.2
Pinocarvone	—	+1.1	+1.1
Pinocarveol	—	+1.8	+1.8
Chrysanthenone	—	+1.1	+1.1
Myrtenol	—	+0.7	+0.8
α-Campholene aldehyde	—	—	+2.8
Carveol	—	—	+0.6
Odour	Fresh, pine-like	Off odour, slightly minty, turpentine-like	

'—' indicates below detection level; * no β-pinene in sample used.

3.6. Oxidation of α-pinene

Samples of α-pinene, under oxygen at 40 °C in the presence of CuO (0.1%) or with UV irradiation, showed much greater conversions (*ca* 17%) than did those of β-pinene (*ca* 5%) under similar conditions, leading to a broader range of products (Table 6) and the development of a minty, turpentine-like off odour. This greater reactivity could again be associated with more allylic hydrogens (7) in α-pinene than in β-pinene (3).

In contrast to the reactions of β-pinene, a significant portion of the product was α-pinene oxide, derived from C=C oxidation. The formation of a similar range of oxidation products from α-pinene, but under very different oxidation conditions, has been reported (McGraw et al., 1999).

3.7. Oxidation of γ-terpinene

γ-Terpinene was even more susceptible to degradation by UV light and O_2 at 37 °C giving over 60% degradation with *p*-cymene as the major product. In contrast, γ-terpinene was significantly less degraded by oxygen in the presence of CuO (0.1%), giving only 4.3% conversion similar to that of β-pinene (4.5%) and much less than α-pinene (17.5%). The decrease using CuO (0.01%) was significantly reduced to 0.9% and this was even further reduced (0.6% and 0.3%) by the addition of tocopherols and BHA respectively.

The results suggest that γ-terpinene, with its doubly allylic hydrogen atoms, is particularly sensitive to hydrogen atom abstraction initiated by UV light as the resulting radicals have greater resonance stabilisation than those formed from the other terpenes.

4. Conclusion

Storage of samples of lemon oil under a range of conditions emphasised the importance of temperature and the presence of oxygen in promoting degradation. Citral, limonene, β- and α-pinenes and γ-terpinene were the compounds most susceptible to degradation and individual samples of these compounds were studied under more forcing conditions, both in the presence and absence of antioxidants.

Oxidation, promoted by the addition of CuO at 40 °C was studied in an oxygen atmosphere. Under these conditions, citral was relatively stable, especially in contrast to α- and β-pinenes and γ-terpinene, which showed significant conversion to oxidation products. Even when the level of CuO added (0.01%) was comparable to that which could be present in water transferred in Cu plumbing, this low level still significantly promoted the oxidation of α-pinene. Addition of the commercial antioxidants, BHA (synthetic) and α-tocopherol (natural), generally led to reduced degradation. BHA was slightly better for protecting γ-terpinene and the antioxidants were the least effective in preventing oxidation of β-pinene. Significantly for lemon oil, both antioxidants inhibited the oxidation of citral.

The amount of degradation, resulting from exposure of citral and the pinenes to UV light for 48 h at 37 °C under oxygen, was very similar to that obtained in the CuO (0.1%) experiments, although a slightly different product mix was obtained in all cases. Citrals were surprisingly stable under both sets of conditions, with less than 1% of oxidation under both conditions. Even this small change, however, led to a detectable loss of lemon odour freshness.

Limonene and γ-terpinene, in contrast to the other compounds, were both much more sensitive to UV degradation, with γ-terpinene showing a major conversion (>60%) to *p*-cymene.

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