COMPOSITIONAL CHANGES IN COMMERCIAL LEMON ESSENTIAL OIL FOR AROMATHERAPY

MASAYOSHI SAWAMURA, U-SUN SON, HYANG-SOOK CHOI, MIE-SOON LEE KIM, NGUYEN THI LAN PHI, MICHAEL FEARS AND CHIZU KUMAGAI

M. Sawamura

Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

Tel.:+81-088-864-5184;

Fax:+81-088-864-5200;

E-mail: sawamura@cc.kochi-u.ac.jp

ABSTRACT

Compositional change in commercial lemon essential oil for application in aromatherapy was investigated under four different conditions over a one-year period. Sample S-1 was stored in an incubator set at 25ºC with the cap open for 3 min everyday; sample S-2 was stored in an incubator set at 25ºC with the cap open once a month for analysis; samples S-3 and S-4 were stored at 5ºC in the same manner as S-1 and S-2, respectively. The oil samples were analyzed quantitatively using an internal standard by GC and GC-MS. Compositional changes occurred predominantly in S-1, where monoterpene hydrocarbons decreased from 97.1% to 30.7% in one year. The increase of *p*-cymene was associated with the decrease of γ-terpinene and citral. The compositional change in S-4 was minimal, while the aerobic condition (S-3) caused some decrease of the constituents during storage even at 5ºC.

INTRODUCTION

Nowadays, we are exposed to a great number of scented products not only in foodstuffs but in many aspects of our daily life, for example in medicines, cosmetics and household products. As a result, it has become more important to study the functions and reactions of fragrances and flavourings in order to ensure the safety of human beings (Song, 2000; Anna et al., 2002; Betty, 2002). Various products incorporating essential oils have been used in aromatherapy for relieving bodily and mental distresses. When a complex of aroma compounds in an essential oil is absorbed by breathing and/or by skin contact, it may relax or stabilize some physical or psychological conditions. The main class of substances used in aromatherapy is essential oil, which is obtained from various aromatic parts of plants by different methods such as steam distillation or cold pressing. It has been known for some time, as reviewed by Susan (1996), that essential oils possess various functional properties such as insect/animal attraction, repellecy effects against insects and animals, inhibitory effects against microorganisms (Morris et al., 1979) and anti-carcinogenic effects. Various citrus oils have been applied in many products such as foods, beverages, cosmetics and medicines as flavoring agents as well as for aromatherapy (Walton, 1984; Salunkhe and Kadam, 1995). Citrus oils are a mixture of volatile compounds and consist mainly of monoterpene hydrocarbons (Shaw, 1979; Sawamura, 2000) which possess high levels of unsaturation and are generally unstable due to many factors such as light, heat, oxidation and hydration (Walton, 1984; Tisserland and Balacs, 1995; Choi and

Sawamura, 2000). Citrus essential oils, on the other hand, generally contain trace amount of coumarins or furanocoumarins (Dellacassa et al., 1997), and anti-oxidants such as flavonoids (Miyake et al., 1997) and tocopherols (Waters et al., 1976) in the non-volatile or volatile fractions of citrus oils. Coumarins and furanocoumarins may have an important role to play in the identification and the quality control of essential oils in addition to pharmacological use (Dugo et al., 1998). Today, a great number of essential oil products for aromatherapy are commercially sold in new age and other high street shops, and the oil products are used in many everyday situations for treatment with essential oils. In order to prevent oxidation nitrogen is put in the bottle and anti-oxidants might be added to the essential oil products by some manufacturers. There is still little chemical information, however, on compositional changes during storage and/or usage (Njoroge et al., 1996; Choi and Sawamura, 2000). It is important for consumers and other parties concerned with aromatherapy to ensure the effectiveness and quality of essential oil products, in addition to guaranteeing their safety. The present study was designed to elucidate the compositional change of commercial lemon essential oil for aromatherapy in consideration of practical applications.

EXPERIMENTAL

Materials

Commercial lemon (*Citrus limon* Burmann) essential oil for aromatherapy was obtained from an aroma products company, Bay House Aromatics,

England, in December 2001. The essential oil was extracted by cold pressing in Murcia, Spain, in May 2001, and stored in 45-gallon steel drums with nitrogen.

Storage condition

Two ml of the essential oil mixed with methyl myristate as internal standard in the ratio of 150:1 were placed in a 4-ml brown vial (Maruem Co., Osaka) of which inner seal of the cap is made from Teflon® to minimize possible migration of chemicals from the bottle closure. The internal standard should be initially put into the sample to compensate for reduction of volume by periodical samplings. The samples were stored in the dark under four different conditions as follows:

S-1: stored in an incubator set at 25°C with the cap being opened for 3 min everyday.

S-2: stored in an incubator set at 25°C with the cap opened for 3 min once a month.

S-3: stored in a refrigerator set at 5°C with the cap opened for 3 min everyday.

S-4: stored in a refrigerator set at 5°C with the cap opened for 3 min once a month.

The incubator used above was a PIC-101 cool incubator (As One Co., Tokyo). The cap opening was done in a laboratory room and the incident light onto the surface of samples was less than 100 lx. This experiment was carried out over a one-year period from December 20, 2001 to December 19, 2002.

Pre and post check of anti-oxidants

The essential oil samples were tested at the outset and the end (12^{th} month) regarding anti-oxidants such as tocopherols and BHA by HPLC according to the previous method (Sawamura et al., 1988).

GC and GC-MS

The essential oil was analyzed using a Shimadzu gas chromatograph GG-14A with a DB-Wax column (60 m × 0.25 mm i.d., film thickness of 0.25 μm; J & W Scientific, Folsom, CA, USA) equipped with a flame ionization detector. The peaks were integrated by a Shimadzu C-R6A Chromatopack integrator. The column temperature was programmed from 70°C (2 min) to 230°C (20 min) at a rate of 2°C/min. Both injector and detector temperatures were 250°C. Nitrogen gas was used as carrier gas at a flow rate of 2 ml/min. The split ratio was 1:50. The oil samples were directly injected to GC and GC-MS. The injection volume into GC was 0.5 μl. A Shimadzu GC-17A coupled with a QP-5000 mass spectrometer was used for GC-MS. The GC condition was the same as that of the GC-14A. Ionization voltage was 70 eV and the ion source temperature was 250°C. The split ratio was 1:32 and the sample size was 0.2 μl. Identification was made by comparison with the MS library of NIST and retention indices on DB-Wax (mentioned above) and DB-1 (60 m × 0.25 mm i.d., film thickness of 0.25 μm; J & W Scientific, Folsom, CA, USA) columns. To make more precise identification, a co-injection method with authentic chemical compounds were additionally employed for several components. The

results were expressed as w/w % on the basis of the internal standard method. The measurement was performed in triplicate. The calculation formula for weight percent is as follows:

$$\text{Amount (w/w\%)} = F_X \times W_S \times (A_X/A_S \times W_E) \times 100$$

where, F_X: response factor for compound X, W_S: weight of internal standard, W_E: weight of essential oil, A_S: peak area of internal standard, A_X: peak area of compound X.

RESULTS AND DISCUSSION

Original Sample

Sixty-four volatile compounds were detected in the original lemon essential oil before storage and sixty were identified, as shown in Table 1. The oil

Table 1

was composed of terpene hydrocarbons (97.1%), aldehydes (1.7%), alcohols (0.6%) and esters (0.3%). Limonene was the most predominant component, accounting for 68.5%, similar to the results obtained in other research on lemon oil. The proportion of β-pinene and γ-terpinene was 12.2% and 7.2%, respectively, which was the same level as previously reported for cold-pressed lemon oil from California and Arizona (Staroscik and Wilson, 1982). Sabinene, α-pinene and myrcene were present in relatively high concentrations, 2.0%, 1.8% and 1.4 %, respectively. The level of *p*-cymene is commonly as much as 0.03%-0.2% on the basis of relative peak area in several varieties of fresh lemon oils (Staroscik and

Wilson, 1982; Njoroge et al., 1994; Sawamura, 2000), while it accounted for up to 1.6% (w/w) in the original sample. It has been reported that *p*-cymene in citrus essential oils increased during storage (Walton, 1984; Njoroge et al., 1996; Choi and Sawamura, 2000). It is difficult to avoid contact with oxygen in the process of cold pressing, even though traders pay attention to storing under nitrogen to prevent oxidation. It is considered that the greater level of *p*-cymene is one of the characteristics of commercial citrus essential oils. It is therefore supposed that this lemon oil product might have been either self-aged or blended with aged oils. Citral (a mixture of neral and geranial) is the most important character-impact odour aldehyde of lemon oil, and its level has been used as index for the assessment of lemon oil quality (Birch et al., 1977; Ichimura and Uchiyama, 1989; Fennema, 1996; Sawamura, 2000). Furthermore, it is reported that the combination of 4-5% citral and fatty aldehydes of C_7 to C_{13} is important basic character compounds of lemon oil (Jensen, 2004). The level was 1.4%, comparable to that in previous reports (Shaw, 1979; Staroscik and Wilson, 1982; Njoroge et al., 1994; Ayedoun and Sossou, 1996; Sawamura, 2000; Vekiri et al., 2002). Eighteen alcohols were detected, but this group was detected in a proportion as low as 0.6%. Two esters, neryl and geranyl acetate, were detected in the original lemon oil, in proportions of 0.08% and 0.24%, respectively. These esters are also contributory aroma compounds, in addition to neral and geranial. Njoroge et al. (1994) reported that their respective proportions in fresh Lisbon cold-pressed oil were 0.31% and 0.23% by relative peak area, whereas neryl acetate was not detected in several varieties of lemon oils (Sawamura, 2000). *cis*- and

trans-Limonene oxides were quantified to be 0.05 % and 0.07% in the original sample, although they are usually detectable in trace amounts in fresh lemon oils (Sawamura, 2000). Ketones were detected in trace amounts as well.

S-1

The relative compositional changes in commercial lemon essential oil stored in an incubator set at 25°C with the cap opened at given intervals, are presented in Table 1. Some compounds changed drastically and others changed slightly, as seen in Table 1. The behavior of these components during storage showed the same tendency as in the previous experiment, in which cold-pressed lemon oil was stored at 20ºC over one year (Njoroge, 1996). The reduction of the peak of internal standard on gas chromatogram was little seen over one year. It implies that methyl myristate will not be decomposed under the present conditions. The percentages of monoterpene hydrocarbons decreased remarkably from 95.6% to 30.0 %. Limonene, a monoterpene hydrocarbon, which is usually the most abundant compound in lemon oil as well as in other citrus oils (Staroscik and Wilson, 1982; Njoroge et al, 1994; Ayedoun and Sossou, 1996; Sawamura, 2000; Vekiri et al., 2002), decreased remarkably from 68.5% to 20.1% over 12 months. *p*-Cymene, which is known as the main substance causing off-flavor in citrus oil, can be converted from γ-terpinene by oxidation (Walton, 1984; Njoroge, 1996). This conversion may have occurred here, because *p*-cymene increased from 1.6% to 7.1% during 5 months, and instead γ-terpinene decreased from 7.2% to 0.01%. It is also suggested that the formation of *p*-

cymene was involved in the cyclic system of citral (Okamura, 1983; Walton, 1984). The detection of *p*-cymen-8-ol after 3 months is proof of the existence of this mechanism. These oxidative conversion mechanisms are demonstrated in Fig. 1.

Fig. 1

α-Pinene and β-pinene decreased remarkably from 1.8% to 0.6% and from 12.2% to 4.0%, respectively. α-Pinene and β-pinene are oxidatively converted to their hydroperoxides with migration of the double bond in the presence of a photosensitizer via *trans*-pinocarveol and myrtenol, respectively (Newman, 1972). The samples were exposed to light for a while during the sampling for analysis or cap-opening. Actually, *trans*-pinocarveol increased after 5 months, and myrtenol appeared after 2 months, increasing gradually thereafter during further storage. However, the levels of both compounds varied to a small extent, because they could be further converted into hydroperoxides. In addition, other monoterpene hydrocarbons such as sabinene and myrcene decreased considerably over one year, from 2.0% to 0.4% and 1.4% to 0.1%, respectively. α-Phellandrene and α-terpinene disappeared after 2 months of storage, while β-phellandrene and terpinolene were not detected after 9 and 4 months, respectively. The level of sesquiterpene hydrocarbons decreased, but not as much as that of monoterpene hydrocarbons. (*E*)-β-Farnesene decreased to 0.5% for 3 months. α-Bergamotene decreased from 0.4% to 0.2% over one year.

The total level of aldehydes decreased from 1.7% to 0.3% over a one year period, and the level of citral (neral and geranial) decreased from

1.4% to 0.2%. Octanal, one of the three aliphatic aldehydes detected, was quantified as 0.2% in the original sample, but was present in a trace amount after 6 months of storage. The level of alcohols increased noticeably, from 0.6% to 4.8%. *p*-Cymen-8-ol, a characteristic compound of grapefruit (Ichimura and Uchiyama, 1989; Fennema, 1996; Sawamura, 2000), occurred after 3 months of storage. Its conversion into *p*-cymene has already been described above. 1,2-Cyclohexendiol showed a considerable increase, from 0.2% to 0.6% in 6 months, and to 1.3 % in 12 months. The levels of three carveol isomers increased noticeably: *trans*-pino-carveol, from 0.1% to 0.4%; *cis*-carveol, up to 0.9%; and *trans*-carveol, up to 0.4% in one year. As for ketones, *p*-menth-8-one and carvone increased continuously up to 0.6% and 1.0%, respectively. 6-Methyl-5-hepten-2-one changed little. The total level of esters increased from 0.3% to 0.5%: neryl acetate increased to 0.4% in 3 months and decreased to 0.2% over a 6 month period; geranyl acetate was almost stable at 0.2% over 9 months, increasing to 0.3% in 12 months. *cis*- and *trans*-Limonene oxides increased from 0.05% to 0.7% and 0.1% to 0.8 % in 5 months, and then were maintained at levels between 0.7% and 0.8% up to the 12^{th} month. Decanoic acid, one of the newly formed compounds, increased gradually during storage, reaching 0.2% after 12 months. Decanal did not seem to be involved as a precursor of decanoic acid because its level was low and varied little. Eight artifacts which had not been detected in the original lemon oil were formed during storage: *cis*-linalool oxide, 2,6-octadien-1-ol, myrtenol, *p*-cymen-8-ol, tetradecane, decanoic acid, and two unidentified compounds (peaks no. 45 and no. 47). The structure of peak no. 47 was

similar to that of β-caryophyllene according to the NIST Library stored in QP-5000.

The aging of essential oils invariably results in the oxidation and resinification reactions occurring simultaneously. The major factor here is the formation of dimmers, trimers and polymers of monoterpene hydrocarbons and other compounds. It is suggested that these polymerization likely resulted in the occurrence of precipitation during storage.

S-2

The compositional changes of the commercial lemon essential oil under the 4 conditions, S-1, S-2, S-3 and S-4 over one year are shown in Table 2.

Table 2

Not all the compounds detected are shown. Regarding S-1, the data on the 1- and 12-months storage are given for convenience of comparison. The hydrocarbons in the S-2 sample decreased from 97.1% to 72.2% in one year. Monoterpenes diminished from 95.6% to 71.5 %, where β-pinene and myrcene reduced from 12.2% to 9.0% and 1.4% to 0.9%, respectively. α-Phellandrene and α-terpinene were detected up to 9 months, but they were not detected after that. Limonene and γ-terpinene decreased from 68.5% to 51.7% and from 7.2% to 2.7%, respectively. *p*-Cymene, on the other hand, increased from 1.9% to 4.1%. The relationship between γ-terpinene and *p*-cymene was examined in the S-1 experiment. Sesquiterpene hydrocarbons did not change so much as the monoterpenes, whereas (*E*)-β-farnesene decreased largely from 1.0% to 0.5% in 3 months. The total level of

aldehydes decreased from 1.7% to 1.0% in one year. Neral and geranial decreased from 0.5% to 0.3% and from 0.9% to 0.5%, respectively. The alcohols were almost stable for 4 months, then their levels increased to a small extent. *cis*-β-Terpineol, myrtenol and *p*-cymen-8-ol were also detected during storage. The ketones such as *p*-menth-8-one, 6-methyl-5-hepten-2-one, camphor and carvone increased to 0.1%. Neryl acetate increased from 0.1% to 0.4% in a year. The total levels of ketones, esters and oxides increased by 2 times over the levels in the original oil.

S-3

The concentration of hydrocarbons decreased predominantly from 97.1% to 64.9% in one year. In particular, the proportion of monoterpene hydrocarbons decreased significantly, as much as in the S-1 sample. The decrease in individual compounds in one year was as follows: β-pinene, 12.2% to 7.2%; sabinene, 2.0% to 1.1%; and γ-terpinene, 7.2% to 1.0 %. The level of limonene diminished significantly from 68.5% to 47.4%, while *p*-cymene increased noticeably, from 1.6% to 5.5%. The level of aldehydes decreased from 1.7% to 1.3% and that of alcohols was almost stable throughout the year. The total level of ketones, esters and oxides showed a similar behavior to those of the S-2 experiment. It was found that the total level of monoterpene hydrocarbons changed noticeably in the aerobic condition. This provides a suggestion as to how to care for the commercial essential oil in daily use.

S-4

The least compositional change was observed in the S-4 experiment. The proportion of hydrocarbons decreased from 97.1% to 93.8% over a one year period. The proportion of limonene changed from 68.5% to 66.1%, and that of γ-terpinene from 7.2% to 5.2%. The proportion of *p*-cymene doubled in one year, even when the sample was stored at 5ºC. Neral and geranial changed slightly, from 0.5% to 0.4% and 0.9% to 0.7%, respectively. The total level of alcohols increased from 0.6% to 0.9 %.

Comprehensive change

The compositional change of lemon essential oil for aromatherapy was studied under the four different storage conditions similar to those in practical use where the oil would be often exposed to air and light for a while at certain temperatures. S-1 and S-3 may be common conditions for users consuming the oil. The qualitative and quantitative changes in monoterpene hydrocarbons content were greater in S-1 than in S-3. In both conditions limonene, β-pinene, γ-terpinene and *p*-cymene changed remarkably during storage. The level of alcohols also changed significantly. The proportion of hydrocarbons in the S-3 sample changed noticeably, but that of the other compounds changed as little as in the S-2 and S-4 samples. This demonstrates that changes in the composition of lemon essential oil will be accelerated by temperature and contact with atmosphere. In tea tree (*Melaleuca alternifolia*) oil terpinen-4-ol is the richest followed by γ-terpinene, while several percents of *p*-cymene are probably an artifact formed from γ-terpinene by oxidation (Verghese et al., 1996). In lime

(*Citrus aurantifolia*) oil the level of *p*-cymene from the degradation of α- and β-pinene, γ-terpinene and citral is also used as an indicator of aging by trading companies. From the aspect of plant functional properties, there is a net increase in the antimicrobial activity of the oxydized essential oils of conifers, which correlated with an increase in the concentration of oxidized monoterpenes such as carvone and carveol. The kind of monoterpene oxidation could also be possible as a part of the chemical defense system of pine (*Pinus sylvestris* L.) seeds against ageing caused by lipid oxidation (Tammela, 2003).

The total amount of functional groups such as hydrocarbons, aldehydes, alcohols, ketones, esters, oxides and acids quatitated may be another indicator of the quality change of essential oil in addition to the compositional change. As for the S-1 sample, the total amount determined by weight percent analysis using GC went down to 91.3% in one month (Table 1). This suggests that about one tenth of the volatile compounds in the original essential oil was converted to nonvolatile compounds during a period of one month. The rate of decrease of the total amount slowed between the 2nd and the 5th month. After 6 months of storage the total amount was as high as 52.0%. After nine months the amount of non-quantitated compounds or nonvolatile materials overcame that of the volatile materials in the essential oil.

As shown in Table 2, the changes in the volatile compounds in S-2 showed a similar tendency to those in S-3. However, the amount retained in S-2 was 90.8% as compared with 84.6% in S-3 over a 6 month period. After 12 months the nonvolatile substances accounted for about 30% in the

both samples. Sample S-2 was stored under anaerobic conditions at 25ºC, while S-3 was stored in an aerobic condition at 5ºC. These results suggest that, above all, the aerobic factor had a larger effect on conversion than temperature. Sample S-4 almost retained its volatile compounds throughout the one year period.

Wabner (2002) pointed out that peroxides could be formed in essential oils. Dissolved oxygen in water reacts under UV light with water molecules to form hydrogen peroxide which subsequently decomposes to hydroxyl radicals as a strong oxidizing agent. Peroxides can be also formed in essential oils. The highly double bonded compounds in essential oils such as α-terpinene, γ-terpinene, terpinen-4-ol and *p*-cymene can easily be oxidized to peroxides. The formation of peroxides also accelerates the peroxidation in the presence of heavy metals. The commodities of essential oil are generally kept in nitrogen atmosphere. However, there are unavoidable factors due to oxidation in the process of extraction and a bulk scale of storage of essential oil (Wabner, 2002). A rather level of *p*-cymene (1.6%) in the original oil may be due to these reasons.

Tocopherols (α-, β-, γ- and δ-) and BHA were analyzed by HPLC. Only α-tocopherol of the 4 tocopherols was detected. The contents in the original, and samples S-1, S-2, S-3 and S-4 after one year were 1350, 170, 660, 800 and 570 ppm, respectively. BHA was detected only in the original sample at a level of 13 ppm, which disappeared after one year. In the previous study the level of α-tocopherol in cold-pressed lemon oil was 2.6 ppm (Song et al., 2001). These results imply that α-tocopherol in excess of 1000 ppm and a small amount of BHA were preliminary added to this

commercial lemon essential oil. They also suggest that the existence of tocopherol cannot always avoid oxidation of the essential oil components.

The allergic symptom caused by the essential oils rich in terpenes from citrus, pine, juniper, black pepper, cypress, etc., is due to hydroperoxides formed during storage (Tisserland and Balacs, 1995). Oxidized tee tree oil in which peroxides, epoxides and endoperoxides are formed causes skin hypersensitivity (Hausen et al., 1999). α- and β-Pinenes may cause unpleasantness to respiratory organs (Filipsson, 1996). An oxide, 1,8-cineol, often gives children a kind of anesthesia including loss of senses and spasm (Darben et al., 1998). It was once thought that *d*-limonene could be a carcinogenic, but according to the recent studies, it is the oxidized compounds derived from *d*-limonene and other terpenes, that induce cancer (Homburger and Boger, 1968). It is rather reported that *d*-limonene plays the role of protection against the cancer (Crowell, 1997; Gould, 1997; Nakaizumi, et al., 1997). These knowledge and the results in this study suggests the importance of handling of essential oils in use.

REFERENCES

Anna, M. A., Ilaria, C., Flavio, M. and Andrea, S. (2002). Effects of the essential oil from citrus lemon in male and female rats exposed to a persistent painful stimulation. *Behavioural Brain Res.*, 136: 127-135.

Ayedoun, A. M. and Sossou, P. V. (1996). Volatile constituents of the peel and leaf oils of *Citrus limon* L. Burm. f. from Benin. *J. Essent. Oil Res.*, 8: 441-444.

Betty, B. (2002). Fragrance: emerging health and environmental concerns. *Flavour Fragr. J.*, 17: 361-171.

Birch, G. G., Brennan, J. G. and Parker, K. J. (1977). The important volatile flavour components of foods. *Sensory Properties of Foods*, Applied Science Publishers, London: 151-166.

Choi, H. S. and Sawamura, M. (2000). Effect of storage conditions on the composition of *Citrus tamurana* Hort. ex Tanaka (*Hyuganatsu*) essential oil. *Biosci. Biotechnol. Biochem*. 66: 439-443.

Crowell, P. L. (1997). Monoterpenes in breast cancer chemoprevention. *Breast Cancer Res. Treat*., 46: 191-197.

Darben, T., Cominos, B. and Lee C. T. (1998). Tropical eucalyptus oil poisoning. *Austral. J. Dermatology*, 39: 265-267.

Dellacassa, E., Lorenzo, D., Moyna, P., Verzera, A., Mondello, L. and Dugo, P. (1997). Uruguayan essential oils. Part VI. Composition of lemon oil. *Flavour Fragr. J*., 12: 247-255.

Dugo, P., Mondello, L., Cogliandro, E., Cavazza, A. and Dugo, G. (1998). On the genuineness of citrus essential oils. Part LIII. Determination of the composition of the oxygen heterocyclic fraction of lemon essential oils (*Citrus limon* (L.) Burm. f.) by normal-phase high performance liquid chromatography. *Flavour Fragr. J*., 13: 329-334.

Fennema, O. R. (1996). Flavors. *Food Chemistry*, 3rd edn., Marcel Dekker, New York: 723-765.

Filipsson, A. F. (1996). Short term inhalation exposure to turpentine: toxicokinetics and acute effects in men. *Occupational Environ. Med.*, 53: 100-105.

Gould, M. N. (1997). Cancer chemoprevention and therapy by monoterpenes. *Environ. Health Perspect*., 105: 977-979.

Hausen, B., Reichling, J. and Harkenthal, M. (1999). Degradation products of monoterpenes are the sentisizing agents in tea tree oil. *Amer. J. Contact Dermatitis*, 10: 68-77.

Homburger, F. and Boger, E. (1968). The carcinogenecity of essential oils, flavors and spices: a review. *Cancer Res*., 28: 2372-2374.

Ichimura, N. and Uchiyama, T. (1989). Characteristic flavor components in citrus fruits. *Koryo*, No. 164: 73-80.

Jensen, B. (2004). http://hjem.get2net.dk/bojensen/index.html

Miyake, Y., Yamamoto, K. and Osawa, T. (1997). Isolation of eriocitrin (epiodictyol 7-rutinoside) from lemon fruit (*Citrus limon* Burm. f.) and its antioxidative activity. *Food Sci. Technol. Int. Tokyo*, 3: 84-89.

Morris, J. A., Khettry, A. and Seitz, E. W. (1979). Antimicrobial activity of aroma chemicals and essential oils. *J. Amer. Oil Chem. So*c., 56: 595-603.

Nakaizumi, A., Baba, M., Uehara, H., Ishii, H. and Tatsuta, M. (1997). *d*-Limonene inhibits *N*-nitrosobis(2-oxopropyl)amine induced hamster pancreatic carcinogenesis. *Cancer Lett*., 117: 99-103.

Newman, A. A. (1972). The monoterpenes. *Chemistry of Terpenes and Terpenoids*, Academic Press, London: 11-87.

Njoroge, S. M., Ukeda, H., Kusunose, H. and Swamura, M. (1994). Volatile

components of Japanese yuzu and lemon oils. *Fravour Fragr. J.*, 9: 159-166.

Njoroge, S. M. (1996). Studies on The flavour chemistry of sour citrus fruits. *Ph. D. Thesis*, Ehime University, Ehime, Japan.

Njoroge, S. M., Ukeda, H. and Sawamura, M. (1996). Changes in the volatile composition of yuzu (*Citrus junos* Tanaka) cold-pressed oil during storage. *J. Agric. Food Chem.*, 44: 550-556.

Okamura, H. (1983). Lemon. *Koryo*, No. 138: 62-67.

Salunkhe, D. K. and Kadam, S. S. (1995). Citrus. *Handbook of Fruit Science and Technology*, Marcel Dekker, New York: 39-90.

Sawamura, M., Kuriyama, T. and Li, Z. (1988). Rind spot, antioxidative activity and tocopherols in the flavedo of citrus fruits. *J. Hort. Sci*., 63: 717-721.

Sawamura, M. (2000). Volatile components of essential oils of the *Citrus genus*. *Recent Research and Development in Agriculture and Chemistry*, Pandalai, S. G. (ed.). Research Signpost: Trivandrum, 4: 131-164.

Shaw, P. E. (1979). Review of quantitative analyses of citrus essential oils. *J. Agric. Food Chem.*, 27: 246-257.

Song, H. S. (2000). Studies on aroma and functional properties of citrus essential oils. *Ph. D. Thesis*, Ehime University, Ehime, Japan.

Song, H. S., Ukeda, H. and Sawamura, M. (2001). Antioxidative activities of citrus peel essential oils and their components against linoleic acid oxidation. *Food Sci. Technol. Res.*, 7: 50-56.

Staroscik, J. A. and Wilson, A. A. (1982). Seasonal and regional variation

in the quantitative composition of cold-pressed lemon oil from California and Arizona. *J. Agric. Food Chem.*, 30: 835-837.

Susan, C. (1996). How do essential oils work? *Essential Oils*, Aurum Press, London: 10-25.

Tammela, P., Nygren, M., Laakso, I., Hopia, A., Vuorela, H. and Hiltunen, R. (2003). Volatile compound analysis of ageing *Pinus sylvestris* L. (Scots pine) seeds. *Flavour Fragr. J.*, 18: 290-295.

Tisserland, R. and Balacs, T. (1995). *Essential Oil Safety*, Churchill Livingstone, London.

Vekiri, S. A., Protopapadakis, E. E., Papadopoulou, P., Papanicolaou, D., Panou, C. and Vamvakias, M. (2002). Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *J. Agric. Food Chem.*, 50: 147-153.

Verghese, J., Jacob, C. V. and Kunjunni Kartha, C. V. (1996). Indian tea tree (*Melaleuca alternifolia* Cheel) essential oil. *Flavour Fragr. J.*, 11: 219-221.

Wabner, D. (2002). The peroxide value – A new tool for the quality control of essential oils. *Intern. J. Aromatherapy*, 12: 216-218.

Walton, B. S. (1984). The essential oils. *The Biochemistry and Physiology of the Lemon*, University of California Division of Agriculture and Natural Resources, CA: 218-309.

Waters, R. D., Kesterson, J. W. and Braddock, R. J. (1976). Method for determining the α-tocopherol content of citrus essential oils. *J. Food Sci.*, 41: 370-371.