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THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE WORKING WITH NATURAL AROMATIC MATERIALS

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Cropwatch's Bibliography for Autoxidation of Essential Oils & Their Components.

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[To be continuously expanded].

Contents:

General
A-Z list: Anise oil
Artemisia
Carene, *δ-3-*
Caryophyllene, *β-*
Chenopodium oil
Citronellol
Citrus oils
Clove oil
Coriander oil
Fennel oil
Geranial
Geraniol
Hop oil
Laurel leaf oil
Lemon oil
Limonene
Linalol/Linalyl acetate/Lavender oil
Mint oils
Myrrh oil
Oregano oil
Santalene, *α-*
Squalene
Tea`tree oil
Terpinene, *γ-*
Turpentine
Vegetable oils.

N.B. 1, Some publications are entered under more than one heading.

2. Some articles on anti-oxidant or sensitizing properties of essential oils are listed where considered relevant.

General

Bråred-Christensson J., Forsström P., Wennberg A.M., Karlberg A.T. & Matura M. (2009) "Air oxidation increases skin irritation from fragrance terpenes." *Contact Dermatitis.* **60**(1),32-40. Abstract. BACKGROUND: Linalool and limonene are common fragrance terpenes that autoxidize on air exposure. The pure compounds are not allergenic but their oxidation products can cause contact allergy. Little has been investigated regarding the irritancy of oxidized terpenes. AIM: The aim of this study was to investigate the irritating effect of pure and oxidized R-limonene and linalool in concentration series and to study the MNIC (Maximum Non Irritant Concentration) of autoxidized linalool and limonene. PATIENTS/METHODS: Patch testing was performed in dermatitis patients and controls with sequentially diluted concentrations of oxidized and non-oxidized linalool, and oxidized and non-oxidized R-limonene. Readings were made with visual assessment and using laser Doppler imaging. RESULTS: The non-oxidized terpenes were non-irritating in all tested concentrations. Both linalool and especially R-limonene were more irritating after oxidation compared with the pure compounds. No difference in response was seen between dermatitis patients and controls. CONCLUSION: Autoxidation of the fragrance terpenes linalool and R-limonene increases irritation. Oxidized linalool is less irritating than oxidized R-limonene. In this study, we found no advantages in using laser Doppler technique compared with visual assessment.

Bråred-Christensson J., Johansson S., Hagvall L., Jonsson S., Börje A. & Karlberg A.-T. (2008) "Limonene hydroperoxide analogues differ in allergenic activity." *Contact Dermatitis* **59**(6), 344-352. Abstract. Background: The fragrance terpene R-limonene is a very weak sensitizer but forms allergenic oxidation products upon contact with air. Oxidized (ox.) limonene is a frequent cause of contact allergy in clinical testing. Objectives: This study investigates the sensitizing potencies of ox. and non-ox. limonene and of structurally closely related limonene hydroperoxides. The clinical importance of the difference in sensitizing potency of two hydroperoxides in autoxidized limonene was studied. Patients/Methods: Ox. and non-ox. limonene were investigated in the murine local lymph node assay (LLNA). Limonene hydroperoxides were investigated using a modified LLNA involving non-pooled lymph nodes and statistical calculations; patch testing of patients with known contact allergy to ox. limonene was performed. Results: A marked increase in the sensitizing potency of ox. limonene compared with that of pure limonene was observed in the LLNA. One analogue, limonene-1-hydroperoxide, was a significantly more potent sensitizer than the other hydroperoxides and gave more positive test reactions in the allergic patients. Conclusions: The results support that hydroperoxides have a specific reactivity indicating that oxygen-centred radicals are important in hapten–protein complex formation of hydroperoxides. The primary oxidation products of ox. limonene, the hydroperoxides, have an important impact on the sensitizing capacity of the oxidation mixture.

Bråred-Christensson J., Matura M., Backtorp C, Borje A., Nilsson J.L., & Karlberg A.T. (2006). “Hydroperoxides form specific antigens in contact allergy.” *Contact Dermatitis*, **55**(4):230-7. Abstract. Concomitant positive reactions to colophonium, oxidized limonene, and/or oxidized linalool are recorded in patch test studies. The main allergens in these patch test mixtures are hydroperoxides, which form antigens by a radical pathway. Theoretically, concomitant reactions can be explained not only by concomitant sensitization or by true cross-reactions but also by the hydroperoxides acting as oxidizing agents on skin proteins to form non-specific antigens without hapten-protein binding. The aim of this study was to explore concomitant reactions and cross-reactivity patterns among hydroperoxide haptens. We investigated whether individuals allergic to the main allergen in colophonium, 15-hydroperoxyabietic acid, would also react to limonene hydroperoxide or linalool hydroperoxide. Only 1 of 29 individuals reacted to more than 1 hydroperoxide. The cross-reactivity pattern among cumene hydroperoxide, limonene hydroperoxide, 1-(1-hydroperoxy-1-methylethyl) cyclohexene (cyclohexene hydroperoxide), and 15-hydroperoxydehydroabietic acid was investigated in guinea-pigs. No general cross-reactivity was observed. Cross-reactions between cumene hydroperoxide and cyclohexene hydroperoxide show that similarity in the overall structure and the way of antigen formation are needed. Quantum calculations were used to determine the formation energies of the intermediary radicals. We concluded that hydroperoxides form specific antigens and that formation of non-specific antigens is unlikely. The concomitant patch test reactions described in the literature are best explained as a result of multiple sensitizations.

Bråred Christensson J, Matura M, Gruvberger B, Bruze M, Karlberg A-T. (undated) "Oxidized linalool - a significant contact sensitizer." Unpublished Manuscript.

Burfield T. (2004) “Notes on oxidation of oils in aromatherapy.” *Aromatherapy Today* **1**(62), 9-10.

Cal K. (2006) “Skin penetration of terpenes from essential oils and topical vehicles.” *Planta Med* **72**, 311-316.

Chalchat J.C., Garry J.R.C., Michet A., Bastide P. & Malhuret J.R. (1989) “The correlation between chemical composition and antimicrobial activity: IV. Comparison between natural and oxidized essential oils with regard to six microbiological strains.” Details?

Chalchat J.C. *et al.* (2000) "Photochemical hydroperoxidation or terpenes. Antimicrobial activity of alpha-pinene, beta-pinene and limonene hydroperoxides" *J Essentail Oil Research* **12**(1), 125-134.

Chiron F., Chalchat J.C., Garry R.P., Pilichowski J.F. & Lacoste J. (1997) "Photochemical hydroperoxidation of terpenes I. Synthesis and characterization of α-pinene, β-pinene and limonene hydroperoxides." *Journal of Photochemistry and Photobiology A: Chemistry* **111** (1-3), 75-86. Abstract. The

photohydroperoxidation of the stereoisomers of three terpenes ((+) and (–) α-pinene, (+) and (–) β-pinene and (+) and (–) limonene) has been performed by using photocatalysts, such as zinc oxide, or sensitizers, such as anthracene or rose Bengal, supported on cross-linked polystyrene. Hydroperoxides accumulated alone in the first stages of sensitized oxidation but were always associated with alcoholic and carbonyl products in the case of ZnO. Secondary products obtained for longer exposure times in sensitized oxidations were identified by gas chromatography/mass spectrometry and mechanisms for their formation, deriving from the photolysis of parent hydroperoxides, were suggested.

Dharmagunawardena B., Takwale A., Sanders K.J., Cannan S., Rodger A. & Ilchyshyn A. (2002) “Gas chromatography: an investigative tool in multiple allergies to essential oils.” *Contact Dermatitis*, **47**(5):288-292.

Fisher A.A & Dooms-Goossens A. (1976) “The effect of perfume “ageing” on the allergenicity of individual perfume ingredients.” *Contact Dermatitis* **2**(3), 155-159.

Fryklöf L-E.. (1954) "Autoxidation of etheric oils used in pharmacy." *Farmacevtisk Revy*, **53**(17), 317-335.

Fryklöf L-E. (1954). "Autoxidation of etheric oils used in pharmacy." *Farmacevtisk Revy*, **53**(19), 361-374.

Kaloustian J., Mikail C., El-Moselhy T., Abou L,& Portugal H. (2007) "GC-MS analysis of allergens in plant oils meant to cosmetics." OCL **14**(2) Mars-Avril 2007 110-115 Abstract. Cutaneous allergy occurs mainly as a result of the use of domestic products and cosmetics. Some fragrances, present in these products, may contain compounds that are responsible for allergy (allergens). The European Council offered a Directive limiting the level of 26 allergens found in cosmetics.GC-MS technique was used to determine the retention times of 25 allergens, determine detection and quantification limits and make calibration with standard solution of each allergen in concentrations ranging from 10 to 200 mgL–1 (21 allergens) and 50 to 200 mgL–1 (4 allergens). Quantification was performed by the use of 2 internal standards (tetradecane and hexadecane). Seven oils issued from plants were studied by GC-MS. For all of them, the concentration of potential allergens was lower than their minimum detectable level. The alcoholic solution of extracts issued from different samples of oil did not demonstrate the presence of any quantifiable allergen, even when was concentrated 25 times. GC-MS could be a useful technique in the identification and, if necessary, quantification of allergen in ingredients meant to cosmetics.

Karlberg A.T., Basketter D., Goossens A. & Lepoittevin J.P. (1999) "Regulatory classification of substances oxidized to skin sensitizers on exposure to air." *Contact Dermatitis*. **40**(4), 183-8. Abstract. Regulatory classification of substances in the European Union (EU) is intended to identify their hazardous toxicological properties in a formal and harmonized manner. In the regulatory work, a specific chemical with its molecular structure is classified as a skin

sensitizer. This implies that the compound is stable throughout its lifetime. The purpose of the present paper is to discuss the problem of skin sensitizing oxidation/degradation products formed by air exposure of various materials or substances with very low allergenic activity. In regulatory classification work on skin sensitizers, the intrinsic suspectibility of a chemical to air oxidation (autoxidation) should be taken into consideration. We give examples of natural terpenoid materials, but the concept of allergens formed by air oxidation can apply to other materials widely used in industrial products. If a positive classification is made for a substance with a known chemical structure, a note should indicate that the primary chemical structure of the notified substance is not a skin sensitizer, but that (some of) its oxidation products are. Complex mixtures which inevitably contain sensitizing oxidation products should (on the basis of sufficient evidence) be classified as skin sensitizing.

Klein E., Farnow H. & Rojan W. (1965) “Secondary products of the autoxidation of cyclic monoterpenes in essential oils” *Dragoco Report* **5**, 98-102.

Lepoittevin J.P. & Karlberg A.T. (1994) "Interactions of allergenic hydroperoxides with proteins: a radical mechanism?" *Chem Res Toxicol*. **7**(2),130-3. Abstract. 1-(1-Hydroperoxy-1-methylethyl)cyclohexene was synthesized as a model compound for the study of the interaction of 15-hydroperoxyabietic acid-like terpenes with proteins. Two related epoxides, 1-(1-hydroxy-1-methylethyl)-2-oxabicyclo[4.1.0]heptane and 2,2-dimethyl-1-oxaspiro-[2.5]octan-4-ol, were also prepared as reference materials. Treatment of the hydroperoxide with $FeCl_3$ and N alpha-Ac-Cys-OMe led to the formation of the corresponding alcohol and of both epoxides. The allergenic activity of these compounds was tested in guinea pigs using the Freund's complete adjuvant test. The hydroperoxide was found to be a strong sensitizer while both epoxides were found to be inactive at the same doses. The generation of highly-reactive radicals in the epidermis could lead to the formation of antigenic structures, the first step of the allergic contact dermatitis mechanism.

Lepoittevin J.P, Karlberg A.T. & Lezerovich A. (1994) “Determination of peroxide value by conventional difference and difference-derivative spectroscopy.” *J. Am. Oil Chem. Soc.* **62**, 1495-1500.

Nilsson J., Carlberg J., Abrahamsson P., Hulthe G., Persson B.A. & Karlberg A.T. (2008) "Evaluation of ionization techniques for mass spectrometric detection of contact allergenic hydroperoxides formed by autoxidation of fragrance terpenes." *Rapid Commun Mass Spectrom.* **22**(22), 3593-8. Abstract. Hydroperoxides formed by autoxidation of common fragrance terpenes are strong allergens and known to cause allergic contact dermatitis (ACD), a common skin disease caused by low molecular weight chemicals. Until now, no suitable methods for chemical analyses of monoterpene hydroperoxides have been available. Their thermolability prohibits the use of gas chromatography and their low UV-absorption properties do not promote sensitive analytical methods by liquid chromatography based on UV detection. In our study, we have investigated different liquid chromatography/mass spectrometry (LC/MS)

ionization techniques, electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI), for detection of hydroperoxides from linalool and limonene.Flow injection analysis was used to evaluate the three different techniques to ionize the monoterpene hydroperoxides, linalool hydroperoxide and limonene hydroperoxide, by estimating the signal efficacy under experimental conditions for positive and negative ionization modes. The intensities for the species [M+H]+ and [M+H-H2O]+ in positive ionization mode and [M-H]- and [M-H-H2O]- in negative ionization mode were monitored. It was demonstrated that the mobile phase composition and instrumental parameters have major influences on the ionization efficiency of these compounds. ESI and APCI were both found to be appropriate as ionization techniques for detection of the two hydroperoxides. However, APPI was less suitable as ionization technique for the investigated hydroperoxides.

Ruberto G. & Baratta M.T. (2000) "Antioxidant activity of selected essential oil components in two lipid model systems." *Food Chem* **69**(2), 167-174. Abstract. About 100 pure components of essential oils have been tested for their antioxidant effectiveness. The main classes of compounds, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, benzene derivatives, and non isoprenoid components comprising alcohols, aldehydes, ketones, which are the most common constituents of essential oils, have been analysed. Two model systems for the antioxidant efficacy have been used; the first exploiting the thiobarbituric acid reactive species (TBARS) method using egg yolk as oxydable substrate, the second measuring the formation of hydroperoxydienes from linoleic acid in a micellar system, using in both cases 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) as a radical initiator, and α-tocopherol as a reference compound. From a general point of view phenols were confirmed to possess the highest antioxidant activity. In particular some monoterpene hydrocarbons, namely, terpinolene, α- and γ-terpinene showed a significant protective action, whereas among the oxygenated components, beside the aforesaid phenols, allylic alcohols manifested an appreciable activity. Sesquiterpene hydrocarbons and non isoprenoid components subjected to this study showed a low, if any, antioxidant effect. The role of the different model systems and the relationship between structure and antioxidant effectiveness are discussed.

Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M. & Bruni R. (2005) "Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods." *Food Chemistry* **91**, 621–632. Abstract. Eleven essential oils, namely, *Cananga odorata* (Annonaceae), *Cupressus sempervirens* (Cupressaceae), *Curcuma longa* (Zingiberaceae), *Cymbopogon citratus* (Poaceae), *Eucalyptus globulus* (Myrtaceae), *Pinus radiata* (Pinaceae), *Piper crassinervium* (Piperaceae), *Psidium guayava* (Myrtaceae), *Rosmarinus officinalis* (Lamiaceae), *Thymus* x *citriodorus* (Lamiaceae) and *Zingiber officinale* (Zingiberaceae), were characterized by means of GC and GC–MS and evaluated for their food functional ingredient related properties. These properties were compared to

those of *Thymus vulgaris* essential oil, used as a reference ingredient. Antioxidant and radical-scavenging properties were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, b-carotene bleaching test and luminol-photochemiluminescence (PCL) assay. In the DPPH assay, *C. odorata*, *C. citratus, R. officinalis* and *C. longa* showed major effectiveness, with a radical inhibition ranging from 59.6 ± 0.42–64.3 ± 0.45%. In the b-carotene bleaching test, *C. odorata* (75.5 ± 0.53%), *R. officinalis* (81.1 ± 0.57%) and *C. longa* (72.4 ± 0.51%) gave the best inhibition results. Similar results were obtained for the same essential oils in the PCL assay. Antimicrobial properties were obtained on five food-spoilage yeasts: *Candida albicans* ATCC 48274, *Rhodotorula glutinis* ATCC 16740, *Schizosaccharomyces pombe* ATCC 60232, *Saccharomyces cerevisiae* ATCC 2365, *Yarrowia lypolitica* ATCC 16617 . *C. citratus* and *T.* x *citriodorus* were the most effective against the tested strains. Suggestions on relationships between chemical composition and biological activities are outlined.

Smith Pease C.K., Basketter D.A. & Patlewicz G.Y (2003) "Contact allergy: the role of skin chemistry and metabolism." *Clin Exp Dermatol.* **28**(2),177-83. Abstract. Chemical reactivity plays the driving role in the biological processes that result in the induction of allergic contact dermatitis. This paper presents an overview of the chemical basis of allergic contact dermatitis, including the physicochemical parameters governing skin penetration, chemical reaction mechanisms associated with haptenation of skin proteins, (quantitative) structure-activity relationships (Q)SARs for contact allergens and prohaptens/skin metabolism of contact allergens. Despite the complexities and poor understanding of some of the metabolic processes leading to skin sensitization, it is possible to describe some of the relationships between chemical structures and the ability to form covalent conjugates with proteins. This knowledge, which relates chemical structure to a specific endpoint, can be programmed into an expert system. The Deductive Estimation of Risk from Existing Knowledge (DEREK) is one such expert system which is described in further detail.

Wabner D (2002) “The peroxide value – a new tool for the quality control of essential oils.” *Int J. Aromatherapy* **12**, 142-144.

Woeber K. & Krombach M (1969) "Sensitisation from volatile oils (preliminary report)." *Berufsdermatosen* **17**(6), 320-326. Abstract. Four points were noted by the report: a) Essential oils from pine needle, dwarf pine, clove and eucalyptus, and eugenol were patch tested on sensitive individuals. The concentration of essential oils used in patch tests appeared to be of minor importance provided that they were pure. b) Using the same five substances, samples were tested from different suppliers and different areas of cultivation. Regional provenance of essential oils had no great influence on sensitisation power. Good quality and lack of ageing were more important. c) It was found that some components of essential oils were sensitisers, namely carene, phellandrene and eugenol. d) Secondary substances contained in low grade oils (peroxides and resinification products) could act as sensitisers to the oil. Botanical sources were not stated.

A-Z listings:

Anise oils.
Miething H., Seger V. & Hänsel R. (2006) "Determination of photoanethole from a stored essential oil of anise fruits as 4,4-dimethoxystilbene by high performance liquid chromatography-ultraviolet coupling." *Phytotherapy Research* **4**(2), 121-123. Abstract. The identification in made of the so-called photoanethole from a stored essential oil of anise fruits as 4,4-dimethoxystilbene using high performance liquid chromatography with photodiode-array detection.

***Artemisia* spp.**
Singh H.P., Kaur S., Mittal S., Batish D.R. & Kohli R.K. (2009) "Essential Oil of *Artemisia scoparia* Inhibits plant growth by generating reactive oxygen species and causing oxidative damage." *J Chem Ecol.* **35**(2),154-62. Abstract. We investigated the chemical composition and phytotoxicity of the essential oil extracted from leaves of *Artemisia scoparia* Waldst. et Kit. (red stem wormwood, Asteraceae). GC/GC-MS analyses revealed 33 chemical constituents representing 99.83% of the oil. The oil, in general, was rich in monoterpenes that constitute 71.6%, with beta-myrcene (29.27%) as the major constituent followed by (+)-limonene (13.3%), (Z)-beta-ocimene (13.37%), and gamma-terpinene (9.51%). The oil and beta-myrcene were evaluated in a dose-response bioassay under laboratory conditions for phytotoxicity against three weeds-*Avena fatua*, *Cyperus rotundus*, and *Phalaris minor*. A significant reduction in germination, seedling growth, and dry matter accumulation was observed in the test weeds. At the lowest treatment of 0.07 mg/ml Artemisia oil, germination was reduced by 39%, 19%, and 10.6% in *C. rotundus*, *P. minor*, and *A. fatua*, respectively. However, the inhibitory effect of beta-myrcene was less. In general, a dose-dependent effect was observed and the growth declined with increasing concentration. Among the three weeds, the inhibitory effect was greatest on C. rotundus, so it was selected for further studies. We explored the explanation for observed growth inhibition in terms of reactive oxygen species (ROS: lipid peroxidation, membrane integrity, and amounts of conjugated dienes and hydrogen peroxide)-induced oxidative stress. Exposure of *C. rotundus* to Artemisia oil or beta-myrcene enhanced solute leakage, indicating membrane disintegration. There were increased levels of malondialdehyde and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. We conclude that Artemisia oil inhibits plant root growth through generation of ROS-induced oxidative damage.

Carene, *δ-3-*
Edman K. *et al.* (2003)“ Exposure assessment to α- & β-pinene, δ-3-carene and wood dust in industrial production of wood pellets.” *British Occupational Hygiene Society* **42**(3), 219-226. Abstract. The main aim of the study was to measure the exposure to monoterpenes (- and ß-pinene and 3-carene) and wood dust during industrial production of wood pellets and briquettes. Additional aims were to compare the results from wood dust sampled on a filter with real time

measurements using a direct reading instrument and to identify peak exposures to dust. Twenty-four men working at six companies involved in industrial production of wood pellets and briquettes participated in the study. Monoterpenes were measured by diffusive sampling and wood dust was measured as total dust. A data logger (DataRAM) was used for continuous monitoring of dust concentration for 18 of the participants. The sampling time was 8 h. The personal exposure to monoterpenes ranged from 0.64 to 28 mg/m3 and a statistically significant (Kruskal–Wallis test, P = 0.0002) difference in levels of monoterpenes for workers at different companies was seen. In the companies the personal exposure to wood dust varied between 0.16 and 19 mg/m3 and for 10 participants the levels exceeded the present Swedish occupational exposure limit (OEL) of 2 mg/m3. The levels of wood dust during the morning shift were significantly (Mann–Whitney test, P = 0.04) higher compared with the afternoon shift. Continuous registration of dust concentration showed peak values for several working operations, especially cleaning of truck engines with compressed air. For 24 workers in six companies involved in industrial production of wood pellets the personal exposure to monoterpenes was low and to wood dust high compared with the present Swedish OEL and previous studies in Swedish wood industries. Since the DataRAM can identify critical working tasks with high wood dust exposure a reduction in exposure levels could probably be achieved by changes in working routines and by the use of protective equipment.

Caryophyllene, *β*-

Börje A., Sköld M., Matura M. & Karlberg A.-T. "Autoxidation of the fragrance caryophyllene forms contact allergens." *Contact Dermatitis* **50**(3), 191-192. Abstract. This is part of an ongoing effort to investigate how autoxidation affects the sensitzing potential of terpene ‐ based fragrances. We have previously shown that terpenes such as abeitic acid (diterpene), limonene and linalool (monoterpenes) form stable hydroperoxides when oxidized. These hydroperoxides have proved to be strong allergens. Its therefore of special interest to study the connection between formation of hydroperoxides caused by autoxidation of fragrance chemicals during handling and storage and an increased allergenic effect. Objective: To investigate the autoxidation of caryophyllene (sesquiterpene) and study its effect on the sensitizing capacity. Methods: Caryophyllene was exposed to air and the autoxidation was monitored by GC and HPLC. The major oxidation products were isolated and their structure determined. The allergenic activity of pure caryophyllene and its oxidation products was investigated in animal assays and clinical testing. Result: Only 10% of the staring material remained after 20 weeks of air exposure. The major oxidation product was caryophyllene oxide. Substantial amounts of formaldehyde were found in the oxidation mixture. Little or no hydroperoxides were detected in the total oxidation mixture. Caryophyllene oxide and oxidized caryophyllene showed a low sensitizing capacity in animals and very few positive reactions at patch testing. Conclusion: Caryophyllene is easily oxidized at air exposure. A low allergenic effect is observed in both sensitization studies and clinical testing. This is consistent with our earlier findings that the amount of hydroperoxides is important for the allergenic activity of autoxidized terpenes

Gramosa N.V. (1994) *Contribuição ao conhecimento químico de plantas do nordeste:* Capparis flexuosa *&* Estudo químico *da autoxidação do β-cariofileno em óleos essenciais*. MSc thesis printed at UFC, Fortaleza, Ceará, Brazil (1994).

Skold M., Karlberg A.T., Matura M., & Borje A. (2006). "The fragrance chemical beta-caryophyllene - air oxidation and skin sensitization." *Food Chem Toxicol*, **44**(4):538-545. Abstract. Fragrances are common causes of allergic contact dermatitis. beta-Caryophyllene is a sesquiterpene that is used as a fragrance chemical. Analogous to the monoterpenes R-limonene and linalool, it can be expected to autoxidize when air exposed. The aim of the present study was to investigate the autoxidation of beta-caryophyllene and to evaluate the effect on the contact allergenic activity. beta-Caryophyllene started to oxidize immediately when air exposed and after 5 weeks almost 50% of the original compound was consumed. Caryophyllene oxide was found to be the major oxidation product. Hydroperoxides of beta-caryophyllene could not be detected in the oxidation mixture. Caryophyllene oxide was shown to be an allergen of moderate strength and beta-caryophyllene air exposed for 10 weeks showed a weak sensitizing capacity in the local lymph node assay. The study reveals that the allergenic activity of beta-caryophyllene is affected by autoxidation, but to a lesser extent when compared to R-limonene and linalool. The present findings support our results in clinical studies showing oxidized beta-caryophyllene to be a rather rare sensitizer compared to oxidized R-limonene and linalool.

Chenopodium oil

Kiuchi F, Itano Y, Uchiyama N, Honda G, Tsubouchi A, Nakajima-Shimada J, Aoki T. (2002) "Monoterpene hydroperoxides with trypanocidal activity from Chenopodium ambrosioides." *J Nat Prod*. **65**(4), 509-12. Abstract. Four monoterpene hydroperoxides were isolated from aerial parts of *Chenopodium ambrosioides* along with ascaridole (1), the anthelmintic principle of this plant, as anti-trypanosomal compounds. The structures of these monoterpenes were determined to be (-)-(2S,4S)- and (-)-(2R,4S)-p-mentha-1(7),8-dien-2-hydroperoxide (2a and 3a) and (-)-(1R,4S)- and (-)-(1S,4S)-p-mentha-2,8-dien-1-hydroperoxide (4a and 5a) on the basis of spectroscopic methods and chemical correlations. In vitro trypanocidal activities of ascaridole (1) and these hydroperoxides (2a-5a) against epimastigotes of Trypanosoma cruzi were 23, 1.2, 1.6, 3.1, and 0.8 microM, respectively. Fresh leaves of C. ambrosioides also contained isomeric hydroperoxides 6a and 7a, and the content ratio of 2a-7a suggested that these hydroperoxides were formed through the singlet-oxygen oxidation of limonene.

Citronellol

Hostynek J.J., Maibach H.I. (2004b) "Sensitisaton potential of citronellol" *Exog Dermatol* **3**(6), 307-312.

Citrus oils.

Misharina T.A. & Samusenko A.L. (2008). "[Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures]" *Prikl Biokhim*

Mikrobiol. **44**(4), 482-6. Abstract. Antioxidant properties of individual essential oils from lemon (*Citrus limon* L.), pink grapefruit (*Citrus paradise* L.), coriander (*Coriandrum sativum* L.), and clove (*Caryophyllus aromaticus* L.) buds and their mixtures were studied by capillary gas-liquid chromatography. Antioxidant activity was assessed by oxidation of the aliphatic aldehyde hexanal to the carboxylic acid. The lowest and highest antioxidant activities were exhibited by grapefruit and clove bud essential oils, respectively. Mixtures containing clove bud essential oil also strongly inhibited oxidation of hexanal. Changes in the composition of essential oils and their mixtures in the course of long-term storage in the light were studied. The stability of components of lemon and coriander essential oils in mixtures increased compared to individual essential oils.

Ting S.V. & Newhall W.F. (2006) "The Occurrence of a Natural Antioxidant in Citrus Fruit." *J Food Science* **30**(1), 57-63. Abstract. Antioxidant activity was found chiefly in the flavedo of citrus fruit. Of the fruits studied, orange had the highest activity and lime and lemon had almost no active principle. Very little, if any, of the activity was located in other component parts of citrus fruit except in the juice vesicles of Valencia oranges. A macro-manometric apparatus was designed to measure the amount of oxygen absorbed by d-limonene and the degree of inhibition of this oxidation when the active principle was added. Comparisons were made between the extracts of citrus fruit and known concentrations of commercial antioxidants. Chromatographic separation on alumina columns yielded a highly colored, oily fraction rich in antioxidant activity. Thin-layer chromatography, a positive ferric chloride-dipyridyl test, and the nonpolar, nonvolatile nature of the active material indicate that it is possibly a tocopherol.

Waters R.D., Kesterson Ww. & Braddock R.J. (2006) "Method for determining the alpha-tocopherol content of citrus essential oils." *J Food Science* **41**(2), 370-371. Abstract. A simple and reliable method for determining the amount of naturally occurring α-tocopherol in citrus essential oils is presented. The unsaponified oil is streaked directly onto a Silica Gel G TLC plate and developed in a benzene-methanol solution to effect separation of tocopherols. The α-tocopherol band is scraped from the plate and eluted with small volumes of absolute ethanol so that subsequent concentration of the fraction is not necessary. Ferric chloride-bipyridyl reagents are added to the eluent and the absorption at 520 nm is determined using a spectrophotometer. A standard curve is prepared using the same procedure so as to eliminate the need for using correction factors.

Clove oil

Misharina T.A. & Samusenko A.L. (2008). "[Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures]" *Prikl Biokhim Mikrobiol.* **44**(4), 482-6. Abstract. Antioxidant properties of individual essential oils from lemon (Citrus limon L.), pink grapefruit (*Citrus paradise* L.), coriander (*Coriandrum sativum* L.), and clove (*Caryophyllus aromaticus* L.) buds and their mixtures were studied by capillary gas-liquid chromatography. Antioxidant activity was assessed by oxidation of the aliphatic aldehyde hexanal to the carboxylic

acid. The lowest and highest antioxidant activities were exhibited by grapefruit and clove bud essential oils, respectively. Mixtures containing clove bud essential oil also strongly inhibited oxidation of hexanal. Changes in the composition of essential oils and their mixtures in the course of long-term storage in the light were studied. The stability of components of lemon and coriander essential oils in mixtures increased compared to individual essential oils

Coriander oil.
Misharina T.A. & Samusenko A.L. (2008). "[Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures]" *Prikl Biokhim Mikrobiol.* **44**(4), 482-6. Abstract. Antioxidant properties of individual essential oils from lemon (*Citrus limon* L.), pink grapefruit (*Citrus paradise* L.), coriander (*Coriandrum sativum* L.), and clove (*Caryophyllus aromaticus* L.) buds and their mixtures were studied by capillary gas-liquid chromatography. Antioxidant activity was assessed by oxidation of the aliphatic aldehyde hexanal to the carboxylic acid. The lowest and highest antioxidant activities were exhibited by grapefruit and clove bud essential oils, respectively. Mixtures containing clove bud essential oil also strongly inhibited oxidation of hexanal. Changes in the composition of essential oils and their mixtures in the course of long-term storage in the light were studied. The stability of components of lemon and coriander essential oils in mixtures increased compared to individual essential oils

Fennel oil.
Misharina T.A. & Polshkov A.N. (2005) [Antioxidant properties of essential oils: autoxidation of essential oils from laurel and fennel and effects of mixing with essential oil from coriander] *Prikl Biokhim Mikrobiol.* **41**(6), 693-702. Abstract. Changes in the composition of essential oils from the seeds of laurel (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare* Mill., var. *dulce* Thelling) and their mixture with essential oil from coriander were studied by capillary gas-liquid chromatography during storage in the dark and in light. Under these conditions, essential oil of laurel retained its composition for 12 months. Essential oil of fennel was rapidly oxidized in light. However, the rate of its oxidation in the dark was lower. The major component of essential oil of fennel, *trans*-anethol, had a lower antioxidant activity than essential oil of coriander. The mixture of essential oils from laurel and coriander possessed antioxidant properties and strongly inhibited the oxidation of components of the fennel oil.

Geranial.
Hagvall, L., Börje, A., Karlberg, A-T (date?) “Autoxidation of geranial.” Unpublished (?) Manuscript. Referred to in Hagvall’s PhD thesis (2009) above.

Geraniol.
Hagvall L, Bäcktorp C, Svensson S, Nyman G, Börje A, Karlberg A.T. (2007) "Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization." *Chem Res Toxicol*. **20**(5), 807-14. Abstract. Fragrances are common causes of contact allergy. Geraniol (trans-3,7-dimethyl-2,6-octadiene-1-

ol) is an important fragrance terpene. It is considered a weak contact allergen and is used for fragrance allergy screening among consecutive dermatitis patients. Analogous to other monoterpenes studied, such as limonene and linalool, geraniol has the potential to autoxidize on air exposure and form highly allergenic compounds. The aim of the present study was to investigate and propose a mechanism for the autoxidation of geraniol at room temperature. To investigate whether allergenic compounds are formed, the sensitizing potency of geraniol itself, air-exposed geraniol, and its oxidation products was determined using the local lymph node assay in mice. The results obtained show that the allylic alcohol geraniol follows an oxidation pattern different from those of linalool and limonene, which autoxidize forming hydroperoxides as the only primary oxidation products. The autoxidation of geraniol follows two paths, originating from allylic hydrogen abstraction near the two double bonds. From geraniol, hydrogen peroxide is primarily formed together with aldehydes geranial and neral from a hydroxyhydroperoxide. In addition, small amounts of a hydroperoxide are formed, analogous to the formation of the major linalool hydroperoxide. The autoxidation of geraniol greatly influenced the sensitizing effect of geraniol. The oxidized samples had moderate sensitizing capacity, quite different from that of pure geraniol. The hydroperoxide formed is believed to be the major contributor to allergenic activity, together with the aldehydes geranial and neral. On the basis of the present study and previous experience, we recommend that the possibility of autoxidation and the subsequent formation of contact allergenic oxidation products are considered in risk assessments performed on fragrance terpenes..

Hagvall L., Baron J. M., Börje A., Weidolf L., Merk H., Karlberg A-T. (2008) “Cytochrome P450 mediated activation of the fragrance compound geraniol forms potent contact allergens.” *Toxicol. Appl. Pharmacol*. **233**, 308-313. Abstract. Contact sensitization is caused by low molecular weight compounds which penetrate the skin and bind to protein. In many cases, these compounds are activated to reactive species, either by autoxidation on exposure to air or by metabolic activation in the skin. Geraniol, a widely used fragrance chemical, is considered to be a weak allergen, although its chemical structure does not indicate it to be a contact sensitizer. We have shown that geraniol autoxidizes and forms allergenic oxidation products. In the literature, it is suggested but not shown that geraniol could be metabolically activated to geranial. Previously, a skin-like CYP cocktail consisting of cutaneous CYP isoenzymes, was developed as a model system to study cutaneous metabolism. In the present study, we used this system to investigate CYP-mediated activation of geraniol. In incubations with the skin-like CYP cocktail, geranial, neral, 2,3-epoxygeraniol, 6,7-epoxygeraniol and 6,7-epoxygeranial were identified. Geranial was the main metabolite formed followed by 6,7-epoxygeraniol. The allergenic activities of the identified metabolites were determined in the murine local lymph node assay (LLNA). Geranial, neral and 6,7-epoxygeraniol were shown to be moderate sensitizers, and 6,7-epoxygeranial a strong sensitizer. Of the isoenzymes studied, CYP2B6, CYP1A1 and CYP3A5 showed high activities. It is likely that CYP1A1 and CYP3A5 are mainly responsible for the metabolic activation of geraniol in the skin, as they are expressed constitutively at significantly higher

levels than CYP2B6. Thus, geraniol is activated through both autoxidation and metabolism. The allergens geranial and neral are formed via both oxidation mechanisms, thereby playing a large role in the sensitization to geraniol.

Hostynek J.J.. & Maibach H.I. (2004) “Is there evidence that geraniol causes allergic contact dermatitis?” *Exogenous Dermatology* **3**(6). Abstract. The fragrance material geraniol has been cited as a frequent cause of allergic contact dermatitis. A review of the literature shows that when the underlying clinical and experimental data are analysed, a clear cause-effect relationship has infrequently or rarely been established. On the basis of the generally weak sensitizing potential of this substance coupled with its generally low exposure conditions, the prevalence of clinical cases would not be expected to be particularly high. That is not to sat that geraniol is a frequent inducer of type IV allergy in members of the public. It remains to be seen, however, how often such allergy, once established, is responsible for any of the cases of allergic contact dermatitis commonly ascribed in the literature. Indeed, in some cases, patch-test conditions `may not be optimal for differentiating between clinically relevant and irrelevant allergy to geraniol. Because of the numerous publications on geraniol-positive patch-test publications, a future effort to ascertain how many of these represent clinical intolerance is indicated. This will also permit determination of the NOEL (no observed effect level) in patch & use testing.

Karlberg A.T., Bergstrom M.A., Borje A, Luthman K., & Nilsson J.L.G (2008). “Allergic contact dermatitis-formation, structural requirements, and reactivity of skin sensitizers.” *Chem Res Toxicol* **21**(1):53-69. Abstract. Contact allergy is caused by a wide range of chemicals after skin contact. Its clinical manifestation, allergic contact dermatitis (ACD), is developed upon repeated contact with the allergen. This perspective focuses on two areas that have yielded new useful information during the last 20 years: (i) structure-activity relationship (SAR) studies of contact allergy based on the concept of hapten-protein binding and (ii) mechanistic investigations regarding activation of nonsensitizing compounds to contact allergens by air oxidation or skin metabolism. The second area is more thoroughly reviewed since the full picture has previously not been published. Prediction of the sensitizing capacity of a chemical is important to avoid outbreaks of ACD in the population. Much research has been devoted to the development of in vitro and in silico predictive testing methods. Today, no method exists that is sensitive enough to detect weak allergens and that is robust enough to be used for routine screening. To cause sensitization, a chemical must bind to macromolecules (proteins) in the skin. Expert systems containing information about the relationship between the chemical structure and the ability of chemicals to haptenate proteins are available. However, few designed SAR studies based on mechanistic investigations of prohaptens have been published. Many compounds are not allergenic themselves but are activated in the skin (e.g., metabolically) or before skin contact (e.g., via air oxidation) to form skin sensitizers. Thus, more basic research is needed on the chemical reactions involved in the antigen formation and the immunological mechanisms. The clinical importance of air oxidation to activate nonallergenic compounds has been

demonstrated. Oxidized fragrance terpenes, in contrast to the pure terpenes, gave positive patch test reactions in consecutive dermatitis patients as frequently as the most common standard allergens. This shows the importance of using compounds to which people are exposed when screening for ACD in dermatology clinics.

Lepoittevin J.P, Karlberg A.T. & Lezerovich A. (1994) “Determination of peroxide value by conventional difference and difference-derivative spectroscopy.” *J. Am. Oil Chem. Soc.* **62**, 1495-1500.

Matura M., Skold M., Borje A., Andersen K.E., Bruze M, Frosch P., Goossens A., Johansen J.D., Svedman C., White I.R. & Karlberg A.T.. (2005) “Selected oxidized fragrance terpenes are common contact allergens.” *Contact Dermatitis*, **52**(6), 320-328. Abstract. Terpenes are widely used fragrance compounds in fine fragrances, but also in domestic and occupational products. Terpenes oxidize easily due to autoxidation on air exposure. Previous studies have shown that limonene, linalool and caryophyllene are not allergenic themselves but readily form allergenic products on air-exposure. This study aimed to determine the frequency and characteristics of allergic reactions to selected oxidized fragrance terpenes other than limonene. In total 1511 consecutive dermatitis patients in 6 European dermatology centres were patch tested with oxidized fragrance terpenes and some oxidation fractions and compounds. Oxidized linalool and its hydroperoxide fraction were found to be common contact allergens. Of the patients tested, 1.3% showed a positive reaction to oxidized linalool and 1.1% to the hydroperoxide fraction. About 0.5% of the patients reacted to oxidized caryophyllene whereas 1 patient reacted to oxidized myrcene. Of the patients reacting to the oxidized terpenes, 58% had fragrance-related contact allergy and/or a positive history for adverse reaction to fragrances. Autoxidation of fragrance terpenes contributes greatly to fragrance allergy, which emphasizes the need of testing with compounds that patients are actually exposed to and not only with the ingredients originally applied in commercial formulations.

Seo K.A., Kim H., Ku H.Y., Ahn H.J., Park S.J., Bae S.K., Shin J.G. & Liu H.K. (2008).”The monoterpenoids citral and geraniol are moderate inhibitors of cyp2b6 hydroxylase activity.” *Chem-Biol Interact*, **174**(3):141-146. Abstract. Monoterpenes are found in the volatile essence of flowers, plants oils, and herbal medicines. Some are commonly used as food additives and fragrance components, and many are found in cosmetics, soaps, cleaning products, disinfectants, preservatives, and medicines. We have recently discovered a moderate inhibitory effect of borneol and isoborneol toward CYP2B6-catalyzed bupropion hydroxylase activity. Based on that result, we expanded our study to evaluate the inhibitory effects of 22 monoterpenoids on CYP2B6 activity in vitro. Among the monoterpenoids screened, borneol, camphor, cineole, isoborneol, menthol, and perillaldehyde showed slight inhibition of CYP2B6-catalyzed bupropion hydroxylation, displaying greater than 50% inhibition at 50muM. Citral and geraniol strongly inhibited CYP2B6 hydroxylase activity in a competitive manner, with K(i) values of 6.8 and 10.3muM, respectively, which are higher than

the K(i) (1.8muM) of the well-known CYP2B6-selective inhibitor thio-TEPA. These in vitro data indicate that high amounts of these two monoterpenoids might interact with drugs that are metabolized by CYP2B6. The in vivo pharmacokinetics of these compounds should be examined to determine whether the inhibition of CYP2B6 activity by monoterpenoids has clinical relevance.

Ginger oil.
Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. (2004) "Antioxidant properties of gingerol related compounds from ginger." *Biofactors* **21**(1-4), 293-6. Abstract.
Ginger (*Zingiber officinale* Roscoe) shows an antioxidant activity, and we have been engaging to determine the structures of more than 50 antioxidants isolated from the rhizomes of ginger. The isolated antioxidants are divided into two groups; gingerol related compounds and diarylheptanoids. In this study, structure-activity relationship of gingerol related compounds was evaluated. Gingerol related compounds substituted with an alkyl group bearing 10-, 12- or 14-carbon chain length were isolated from the dichloromethane extract of rhizomes using repeated chromatographic techniques. The antioxidant activities of these compounds were evaluated by the following measurements; 1) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, 2) inhibitory effect on oxidation of methyl linoleate under aeration and heating by the Oil Stability Index (OSI) method, and 3) inhibitory effect on oxidation of liposome induced by 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH). These results suggested that the substituents on the alkyl chain might contribute to both radical scavenging effect and inhibitory effect of autoxidation of oils, while inhibitory effects against the AAPH-induced peroxidation of liposome was somewhat influenced by the alkyl chain length; the antioxidant activity might be due to not only radical scavenging activity of antioxidants but also their affinity of the antioxidants to the substrates.

Hop oil
Menary, R.C., Williams, E.A. and Nickerson, G.B. (1986). "Effect of mytenol on the rate of oxidation of alpha- & beta-acids in hops." *Acta Hort.* (ISHS) **188,** 149-156

Laurel leaf oil.
Misharina T.A. & Polshkov A.N. (2005) [Antioxidant properties of essential oils: autoxidation of essential oils from laurel and fennel and effects of mixing with essential oil from coriander] *Prikl Biokhim Mikrobiol.* **41**(6), 693-702. Abstract.
Changes in the composition of essential oils from the seeds of laurel (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare* Mill., var. *dulce* Thelling) and their mixture with essential oil from coriander were studied by capillary gas-liquid chromatography during storage in the dark and in light. Under these conditions, essential oil of laurel retained its composition for 12 months. Essential oil of fennel was rapidly oxidized in light. However, the rate of its oxidation in the dark was lower. The major component of essential oil of fennel, transanethol, had a lower antioxidant activity than essential oil of coriander. The mixture of essential

oils from laurel and coriander possessed antioxidant properties and strongly inhibited the oxidation of components of the fennel oil.

Lemon oil

Ben-Yehoshua S, Rodov V, Nafussi B, Feng X, Yen J, Koltai T, Nelkenbaum U. (2008) "Involvement of limonene hydroperoxides formed after oil gland injury in the induction of defense response against *Penicillium digitatum* in lemon fruit." *J Agric Food Chem*. **56**(6):1889-95. Abstract. The effects of wounding oil glands of lemon [*Citrus limon* (L.) Burm.] fruit were investigated. Young mature-green lemons demonstrated significantly lower decay incidence than older yellow fruit when their oil glands were punctured in the presence of post-harvest wound pathogen *Penicillium digitatum* Sacc. Contact with the released gland content on the green lemon surface reduced the viability of *P. digitatum* spores approximately twice. Wounding caused rapid production of limonene hydroperoxides that persisted for only a few minutes. The magnitude depended on the physiological maturity of the fruit; mature-green fruit produced much higher levels than did yellow lemons. Furthermore, wounding of the oil glands or injection of limonene hydroperoxides into the lemon peel elicited the production of the citrus fruit phytoalexins, scoparone and scopoletin, to levels known to be effective in reducing decay caused by *P. digitatum*. The mature-green fruit produced about twice as much of these phytoalexins as the older yellow fruit. This induced defensive elicitation of phytoalexin

Nguyen H., Campi E.M.,Jackson W.R. & Antonio F. Patti A.F. (2008) "Effect of oxidative deterioration on flavour and aroma components of lemon oil." *Food Chemistry* **112**(2), 388-393. 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.

Schieberle P. & Grosch W.(2005) "Potent odorants resulting from the peroxidation of lemon oil" *Zeitschrift für Lebensmitteluntersuchung und - Forschung A* **189**(1), 1431-4630. Abstract. The odorants of a lemon oil sample, which was peroxidized for 120 h at room temperature in the presence of light, were analysed by an aroma extract dilution procedure. On the basis of their FD factors carvone,p-methylacetophenone,p-cresol, 4-acetyl-1-methyl-1-cyclohexene and 4 hydroperoxides were identified as the most intense odorants. Changes in the concentrations of neral, geranial, linalool (odorants of the fresh lemon oil), carvone,p-methylacetophenone,p-cresol andp-cymene (dependent on

the storage time) were determined and the odour units for these compounds were calculated.

Lemongrass oil

Orafidiya L. O. (2006) "The effect of autoxidation of lemon-grass oil on its antibacterial activity." *Phytotherapy Research* **7**(3), 269-271. Abstract. The effect of autoxidation of lemon-grass oil on its antibacterial activity has been studied. Using the Active Oxygen method, the oil was found to undergo rapid oxidation under accelerated test conditions. The oxidized oil samples were found to have reduced activity against bacteria. This activity was completely lost in extensively oxidized oil samples. Inclusion of antioxidants in the oil samples reduced the rate of oxidation and enhanced the antibacterial activity of the oil. The effects of the antioxidants were concentration dependent, and at their effective concentrations, oxidation was completely prevented for the period of the test.

Limonene

Ben-Yehoshua S, Rodov V, Nafussi B, Feng X, Yen J, Koltai T, Nelkenbaum U.(2008) "Involvement of limonene hydroperoxides formed after oil gland injury in the induction of defense response against *Penicillium digitatum* in lemon fruit." *J Agric Food Chem*. **56**(6):1889-95. Abstract. The effects of wounding oil glands of lemon [*Citrus limon* (L.) Burm.] fruit were investigated. Young mature-green lemons demonstrated significantly lower decay incidence than older yellow fruit when their oil glands were punctured in the presence of postharvest wound pathogen *Penicillium digitatum* Sacc. Contact with the released gland content on the green lemon surface reduced the viability of *P. digitatum* spores approximately twice. Wounding caused rapid production of limonene hydroperoxides that persisted for only a few minutes. The magnitude depended on the physiological maturity of the fruit; mature-green fruit produced much higher levels than did yellow lemons. Furthermore, wounding of the oil glands or injection of limonene hydroperoxides into the lemon peel elicited the production of the citrus fruit phytoalexins, scoparone and scopoletin, to levels known to be effective in reducing decay caused by *P. digitatum*. The mature-green fruit produced about twice as much of these phytoalexins as the older yellow fruit. This induced defensive elicitation of phytoalexin production, as well as the direct effects of these antifungal compounds, markedly inhibited the pathogen in mature-green fruits but was ineffective in older yellow ones.

Bernard R.A. & Marr A.G. (2006) “The oxidation of terpenes. 1. Mechanism & reaction products of d-limonene autoxidation.” *J Food Science* **25**(4), 517-30.

Bråred-Christensson J., Johansson S., Hagvall L., Jonsson C., Börje A. & Karlberg AT. (2008) Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis.* **59**(6),344-52. Abstract. BACKGROUND: The fragrance terpene R-limonene is a very weak sensitizer but forms allergenic oxidation products upon contact with air. Oxidized (ox.) limonene is a frequent cause of contact allergy in clinical testing. OBJECTIVES: This study investigates the sensitizing potencies of ox. and non-ox. limonene and of structurally closely related limonene hydroperoxides. The clinical importance of the difference in

sensitizing potency of two hydroperoxides in autoxidized limonene was studied. PATIENTS/METHODS: Ox. and non-ox. limonene were investigated in the murine local lymph node assay (LLNA). Limonene hydroperoxides were investigated using a modified LLNA involving non-pooled lymph nodes and statistical calculations; patch testing of patients with known contact allergy to ox. limonene was performed. RESULTS: A marked increase in the sensitizing potency of ox. limonene compared with that of pure limonene was observed in the LLNA. One analogue, limonene-1-hydroperoxide, was a significantly more potent sensitizer than the other hydroperoxides and gave more positive test reactions in the allergic patients. CONCLUSIONS: The results support that hydroperoxides have a specific reactivity indicating that oxygen-centred radicals are important in hapten-protein complex formation of hydroperoxides. The primary oxidation products of ox. limonene, the hydroperoxides, have an important impact on the sensitizing capacity of the oxidation mixture.

Johansson S., Giménez-Arnau E., Grøtli M., Karlberg A.T. & Börje A. (2008) "Carbon- and oxygen-centered radicals are equally important haptens of allylic hydroperoxides in allergic contact dermatitis." *Chem Res Toxicol.* **21**(8), 1536-4. Abstract. Limonene is one of the most commonly used fragrance compounds in western countries today. When exposed to air, it autoxidises, forming hydroperoxides that are strong contact allergens. To cause allergic contact dermatitis (ACD), the hydroperoxides are considered to bind covalently to proteins in the skin via a radical pathway. Consequently, the nature and reactions of the radicals formed from the hydroperoxides are important. We have examined the radical formation from, and sensitizing potential of, three allylic hydroperoxides. Two of these are found in the oxidation mixture of limonene, while the third is a synthetic structural analogue. The identity of the radicals formed from these hydroperoxides has been studied in radical trapping experiments. Chemical trapping experiments were performed using 5,10,15,20-tetraphenyl-21 H,23 H-porphine iron(III) chloride [Fe(III)TPPCl 3] as an initiator and 1,1,3,3-tetramethylisoindolin-2-yloxyl as a radical trapper. Electron paramagnetic resonance experiments using photolysis for initiation were performed with and without 5-diethoxy-phosphoryl-5-methyl-1-pyrroline N-oxide. Our results demonstrate the ability of the studied hydroperoxides to form peroxyl, allyloxyl, and oxiranylcarbinyl radicals. These radicals can potentially react with proteins to form immunogenic hapten-protein complexes relevant for ACD. The sensitizing potency of the hydroperoxides was studied in the murine local lymph node assay. All three hydroperoxides were found to be potent sensitizers with some variations, which can be related to the identity and quantity of the radicals formed. The results indicate that both carbon- and oxygen-centered radicals are important intermediates in the formation of hapten-protein complexes and that the sensitizing potency of the hydroperoxides is related to their structures.

Karlberg A.-T. & Dooms-Goossens A. (2006) "Contact allergy to oxidized d-limonene among dermatitis patients." *Contact Dermatitis* **36**(4), 201-206. Abstract. d-Limonene. obtained as a by-product from the citrus juice industry was introduced on the market as a more environmentally friendly defatting and

cleaning agent than the traditionally used organic solvents. Autoxidation of d-limonene readily occurs to give a variety of oxygenated monocyclic terpenes that are strong contact allergens. The aim of the present study was to investigate the prevalence of contact allergy to air exposed d-limonene among dermatitis patients. A fraction consisting of d-limonene hydroperoxides was also tested. Screening with oxidized d-limonene will detect cases of allergic contact dermatitis. Additional cases were detected when testing with the fraction of limonene hydroperoxides. The proportion of positive patch test reactions to oxidized d-limonene was comparable to that seen for several of the allergens within the standard series. An increased UAT of d-limonene containing allergenic oxidation products in industry where high concentrations are used, as well as in domestic exposure, might result in contact sensitization and dermatitis. Patients reacting to d-limonene often reacted to fragrance mix, balsam of Peru and colophony in the standard series.

Karlberg A.T., Magnusson K. & Nilsson U. (1992) "Air oxidation of d-limonene (the citrus solvent) creates potent allergens." *Contact Dermatitis*. **26**(5), 332-40. Abstract. Products containing as much as 95% of d-limonene are used for, e.g., degreasing metal before industrial painting and for cleaning assemblies. Experimental studies on the sensitizing potential of limonene show diverging results. In a previous study, we found that the sensitizing potential of d-limonene increased with prolonged air exposure. The aim of this study was to make further chemical analyses, to identify compounds formed by air exposure of d-limonene and to study their allergenic potential. d-limonene was found to be a sensitizer after prolonged exposure to air according to 2 Freund's complete adjuvant test (FCAT) experiments and 1 guinea pig maximization test (GPMT) study. No significant response was obtained to d-limonene not air exposed, even if the animals were sensitized to oxidized d-limonene. 5 main oxidation products of d-limonene were identified. (R)-(-)-carvone and a mixture of cis and trans isomers of (+)-limonene oxide were found to be potent sensitizers, while no significant reactions were obtained in the animals induced with a mixture of cis and trans isomers of (-)-carveol. It can be concluded that air oxidation of d-limonene is essential for its sensitizing potential, and that potent allergens are created.

Karlberg A.T., Magnusson K. & Nilsson U. (1994) “.Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene.” *Ann Occup Hyg*, **38**(2):199-207.

Karlberg A.T., Shao L.P., Nilsson U., Gäfvert E. & Nilsson J.L.(1994) "Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch Dermatol Res*. **286**(2),97-103. Abstract. Hydroperoxides of d-limonene were shown to be potent contact allergens when studied in guinea-pigs. Limonene-2-hydroperoxide (2-hydroperoxy-p-mentha-6,8-diene, a mixture of trans and cis isomers) was synthesized for the first time. The ratio between the trans and cis forms was 3:1. These two hydroperoxides were identified as the major hydroperoxides in autoxidized d-limonene. In photo-oxidized d-limonene, they constituted a minor part of the hydroperoxide fraction. Hydroperoxides may bind

to proteins of the skin to make antigens either via a radical mechanism or after reactions to give epoxides. The cross-reactivity between the epoxide limonene-1,2-oxide, a potent contact allergen, and the hydroperoxides was therefore studied. No significant pattern of cross-reactivity was found. Further studies to identify and test the allergenicity of single hydroperoxides are needed to elucidate the mechanism of the allergenicity.

Karlberg A.-T., Boman A & Melin B.(1991) "Allergenicity of d-limonene - the citrus solvent." *British Occupational Hygiene Society* **35**(4), 419-426. Abstract. With the increasing use of d-limonene as a substitute for chlorinated hydrocarbons, chlorofluorocarbons (CFC) and other organic solvents, a demand has arisen for more knowledge of the health effects of this substance. The aim of the present study was to investigate the allergenic effect of d-limonene on skin. The sensitizing potential of d-limonene and the influence of air exposure on its allergenicity were studied in guinea-pigs. d-Limonene of high purity gave no significant allergic reactions, while d-limonene exposed to air for 2 months sensitized the animals. Gas chromatographic analyses indicated that the content of limonene oxide in limonene increased with prolonged air exposure. It is concluded that allergenic compounds are formed from d-limonene upon prolonged air exposure. Products containing d-limonene should therefore be kept in cold storage in closed vessels. Manual handling should be avoided, or gloves worn, in order to reduce the risk of sensitization.

Karlberg A.-T., Magnusson K. & Nilsson U. (1991) "Influence of an anti-oxidant on the formation of allergenic compounds during the auto-oxidation of d-limonene." *British Occupational Hygiene Society* **38**(2), 199-2017. Abstract. Butylated hydroxytoluene (BHT), a common anti-oxidant, was added to different samples of *d*-limonene The decrease in concentration of *d*-limonenc and the formation of oxidation products were compared between the samples and with samples without anti-oxidant using gas chromatography. The aim of the study was to investigate how long *d*-limonene to which BHT was added could be handled when air-exposed at room temperature, without formation of oxidation products which according to previous studies increase the risk of skin sensitization. In expenments trying to mimic the handling of limonene products at workplaces the addition of BHT prevented auto-oxidation for periods depending on the purity of the products and on the room temperature. Cold and dark storage of *d*-limonene in closed vessels prevented auto-oxidation for 1 year without addition of anti-oxidant.

Li T.H., Turpin B.J., Shields H.C. & Weschler CJ. (2002) "Indoor hydrogen peroxide derived from ozone/d-limonene reactions." *Environ Sci Technol*. **36**(15), 3295-302. Abstract. In this pilot study, performed in an office manipulated to resemble an environment with a strong indoor ozone source or a significant influx of outdoor air during a smog event, reactions between ozone and d-limonene produced hydroperoxides. Hydrogen peroxide (H202) presumably constituted the majority of the measured hydroperoxides, although a small amount of organic hydroperoxides (ROOH) may have contributed to the signal. Total

hydroperoxides were 1.0-1.5 ppb at low air exchange rates (0.5-4 h(-1)) and 0.6-0.8 ppb at high air exchange rates (12-18 h-1). The net estimated yield ranged from 1.5 to 3.2%, consistent with values reported in the literature. Based on these yields and typical indoor scenarios, peak indoor concentrations of H202 are projected to be comparable with, but not significantly larger than, peak outdoor concentrations. Hygroscopic secondary organic aerosols (SOA; 10-100 microg m(-3)) were simultaneously generated by the ozone/d-limonene reactions; their co-occurrence with H202 provides a mechanism whereby H2O2 can be transported into the lower respiratory tract. The results demonstrate that reduced air exchange rates lead to increased concentrations of H2O2 and SOA as well as a shift in the size-distribution toward larger particles (0.3-0.7 microm diameter), potentially increasing the amount of H2O2 delivered to the lower respiratory region. This study increases our understanding of H2O2 exposures, including exposures to H2O2 associated with co-occurring hygroscopic aerosols. It also re-emphasizes the potential of ozone-driven chemistry to alter indoor environments, often producing products more irritating than their precursors.

Matura M., Goossens A., Bordalo O., Garcia-Bravo B., Magnusson K., Wrangsjö K., & Karlberg A.T. (2002) "Oxidized citrus oil (R-limonene): a frequent skin sensitizer in Europe." *J Am Acad Dermatol.* **47**(5), 709-14. Abstract. BACKGROUND: Peel oil from citrus fruits consists of R-(+)-limonene, which is one of the most commonly used fragrance materials in technical products and in fine fragrances. This substance forms allergenic oxidation products during handling and storage. OBJECTIVE: We wanted to study the frequency of allergic reactions to oxidized R-(+)-limonene in patients with dermatitis and find a suitable test preparation. METHOD: Patch testing with oxidized R-(+)-limonene was performed on 2273 patients at 4 dermatology clinics in Europe. RESULTS: Of the consecutive patients tested, 3.8% to 3.9% had positive reactions in two of the clinics; 6.5% had positive reactions in the third clinic; and 0.3% had positive reactions in the fourth clinic. A total of 63 patients showed positive reactions. In total, 57% of the patients did not react to fragrance mix or balsam of Peru. We recommend testing with 3% oxidized R-(+)-limonene in patients referred for patch testing. CONCLUSION: The high frequency of oxidized limonene allergy provides clinical evidence for the European classification of R-(+)-limonene that contains oxidation products as skin sensitizers.

Matura M., Goossens A., Bordalo O., Garcia-Bravo B., Magnusson K., Wrangsjo K. & Karlberg A.T. (2003). “Patch testing with oxidized r-(+)-limonene and its hydroperoxide fraction.” *Contact Dermatitis* **49**(1), 15-21. Abstract. R-(+)-Limonene is an ubiquitous allergen in our environment. It is one of the most widely used fragrance materials not only in fine fragrances but also most often incorporated in domestic and occupational products. Although the non-oxidized R-(+)-limonene itself is not allergenic, it easily forms allergenic products due to autoxidation during handling and storage. 2273 patients at 4 dermatological clinics in Europe were patch tested between 1997 and 1999 in 2 steps. First, the oxidation mixture of R-(+)-limonene and 1 selected allergen fraction of the mixture, the limonene hydroperoxides, were tested in 2 different vehicles in

consecutive patients. A diverging frequency of positive patch test reactions was observed in the 4 clinics. 3.8% of the consecutive patients tested reacted to oxidized R-(+)-limonene in 2 clinics, 6.5% in the 3rd, whereas 0.3% in the 4th clinic. In 2 of the centres, different but significant concomitant positive response rates to other allergens were observed; e.g. to fragrance materials and to colophonium. However, in the total test population, 57% of the limonene-allergic subjects did not react to any of the fragrance allergy markers used in the standard series. In the 2nd step, patients showing positive reactions were retested, also including additional separate allergens of the limonene oxidation mixture (carvone and limonene oxide). 60% of the limonene-allergic patients showed positive results at retesting. The limonene hydroperoxide fraction was proved to be the most important allergen of the oxidation mixture, showing positive reactions in around 60% of the limonene-allergic patients at both test sessions. Testing limonene oxide and carvone separately resulted in very few positive reactions. 3% oxidized R-(+)-limonene in non-stabilized petrolatum is most suitable when using only 1 test preparation for diagnosis of contact allergy to oxidized limonene. Our data give clinical support to the European classification of R-(+)-limonene, containing oxidation products, as a skin sensitizer.

Matura M, Sköld M, Börje A, Andersen KE, Bruze M, Frosch P, Goossens A, Johansen JD, Svedman C, White IR, Karlberg AT. (2006) "Not only oxidized R-(+)- but also S-(-)-limonene is a common cause of contact allergy in dermatitis patients in Europe." *Contact Dermatitis* **55**(5), 274-9. Abstract. Limonene, one of the most often used fragrance terpenes in any kind of scented products, is prone to air-oxidation. The oxidation products formed have a considerable sensitizing potential. In previous patch test studies on consecutively tested dermatitis patients, oxidized R-limonene has been proven to be a good and frequent indicator of fragrance-related contact allergy. The current study extends these investigations to 6 European clinics of dermatology, where the oxidation mixture of both enantiomers of limonene (R and S) have been tested in 2411 dermatitis patients. Altogether, 63 out of 2411 patients tested (2.6%) reacted to 1 or both the oxidized limonene preparations. Only 2.3% reacted to the oxidized R-limonene and 2.0% to the oxidized S-limonene. In 57% of the cases, simultaneous reactions were observed to both oxidation mixtures. Concomitant reactions to the fragrance mix, colophonium, Myroxylon pereirae, and fragrance-related contact allergy were common in patients reacting to 1 or both the oxidized limonene enantiomers. Our study provides clinical evidence for the importance of oxidation products of limonene in contact allergy. It seems advisable to screen consecutive dermatitis patients with oxidized limonene 3% petrolatum, although this patch test material is not yet commercially available.

Nilsson U., Magnusson K., Karlberg O., & Karlberg A.T.. “Are contact allergens stable in patch test preparations? Investigation of the degradation of d-limonene hydroperoxides in petrolatum. *Contact Dermatitis* **40**(3):127_32, 1999. Abstract. Several of the products formed after oxidation of d-limonene exhibit strong contact allergenic properties. Some, e.g., the hydroperoxides, are unstable compounds. In this study, we have examined whether the limonene

hydroperoxides are chemically stable in white petrolatum used for patch testing. We found that the stability of the hydroperoxides was strongly dependent on whether or not the petrolatum was stabilized with α-tocopheryl acetate. In the presence of this antioxidant, the hydroperoxides were degraded to a greater extent. The hydroperoxides were shown to be directly reduced to the corresponding alcohols by this agent. On the other hand, the compounds where shown to be stable in non-stabilized petrolatum throughout clinical patch testing for a period of 6 weeks, provided that the preparations were stored in a refrigerator when not used. Thus, it is recommended that vehicles without α-tocopheryl acetate are used when peroxy or hydroperoxy compounds are patch tested or used in sensitization experiments. However, it is important to limit the storage time so that optimal conditions are at hand. A fast method was developed to enable isolation and quantification of the hydroperoxides in white petrolatum. This analytical method may also be applicable to other compositions of patch test preparations.

Taher H.A. & Ubiergo G.O. (1987) "[The autoxidation of limonene at moderate oxygen pressure]" *Essenze Derivati Agrumari* **54** (2) 122-127

Linalol / **Linalyl acetate** / **Lavender oil**

Bäcktorp C., Wass J.R., Panas I., Sköld M., Börje A. & Nyman G. (2006) "Theoretical investigation of linalool oxidation." *J Phys Chem A*. **110**(44), 12204-12. Abstract. This study concerns the autoxidation of one of the most used fragrances in daily life, linalool (3,7-dimethyl-1,6-octadien-3-ol). It reacts with O2 to form hydroperoxides, which are known to be important contact allergens. Pathways for hydroperoxide formation are investigated by means of quantum mechanical electronic structure calculations. Optimized molecular geometries and harmonic vibrational frequencies are determined using density functional theory (DFT). Insight into how the addition of O2 to linalool occurs is obtained by establishing a theoretical framework and systematically investigating three smaller systems: propene, 2-methyl-2-butene, and 2-methyl-2-pentene. 2-Methyl-2-pentene was chosen as a model system and used to compare with linalool. This theoretical study characterizes the linalool-O2 biradical intermediate state, which constitutes a branching point for the further oxidation reactions pathways. Thus, the observed linalool oxidation product spectrum is discussed in terms of a direct reaction path, the ene-type mechanism, and the radical mechanism. The major hydroperoxide found in experiments is 7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol, and the calculated results support this finding.

Bråred Christensson J, Matura M, Gruvberger B, Bruze M, Karlberg A-T. (undated) "Oxidized linalool - a significant contact sensitizer." Unpublished Manuscript.

Basketter D.A., Wright Z.M., Colson N.R., Patlewicz G.Y. & Pease C.K.: (2002) “Investigation of the skin sensitizing activity of linalool.” *Contact Dermatitis* 2002; **47**: 161–164. Abstract. An increasing range of chemicals appears to be capable of causing skin sensitization as a result of their capacity to undergo air oxidation (autoxidation) with the consequent formation of reactive species such as

epoxides and hydroperoxides. In this small investigation, the ability of linalool, a common fragrance ingredient, to cause such effects was quantified using the local lymph node assay before and after careful purification by vacuum distillation. The commercially available grade of linalool (97% purity) was shown to be a weak skin sensitizer. Various impurities, including linalool oxide, dihydrolinalool, epoxylinalool, 3-hexenyl butyrate and 3,7-dimethyl-1,7-octadiene-3,6-diol were identified and were completely removed (except for the dihydrolinalool remaining at 1.4%) and the re-purified linalool retested. Neither linalool or dihydrolinalool are protein-reactive compounds. The sensitization potency of the re-purified linalool sample was considerably reduced, but not entirely eliminated, suggesting either that an allergenic impurity could be very quickly reformed by mechanisms of activation or that certain potent undetectable allergens remained. Both possibilities are consistent with what is understood of the chemistry and composition of commercially available linalool.

Bezard M., Karlberg A.T., Montelius J., &. Lepoittevin J.P (1997) “Skin sensitization to linalyl hydroperoxide: support for radical intermediates.” *Chem Res Toxicol*, **10**(9):987-93, Abstract. Few studies are reported on the formation of reactive carbon-centred radical species from toxic xenobiotics. In this paper the formation of carbon radicals derived from the skin sensitizer linalyl hydroperoxide is described using radical trapping and EPR studies. Radical trapping used TMIO as scavenger agent and light, heat or TPP-Fe(3+) as radical inducers. EPR spin trapping was based on the use of the parent alcohol, generating the same allyloxyl radical than the hydroperoxide by photolysis of the corresponding nitrite formed with t-BuONO, also playing the role of the spin trap. It is suggested that the generation of these carbon radical species could play an important role for the binding of the hydroperoxide with skin proteins to form antigenic structures, the first step of the skin sensitization mechanism.

Bezard M., Giménez-Arnau E., Meurer B., Grossi L., Lepoittevin J.P. (2005) "Identification of carbon-centred radicals derived from linalyl hydroperoxide, a strong skin sensitizer: a possible route for protein modifications." *Bioorg Med Chem.* **13**(12),3977-86. Abstract. Few studies are reported on the formation of reactive carbon-centred radical species from toxic xenobiotics. In this paper the formation of carbon radicals derived from the skin sensitizer linalyl hydroperoxide is described using radical trapping and EPR studies. Radical trapping used TMIO as scavenger agent and light, heat or TPP-Fe(3+) as radical inducers. EPR spin trapping was based on the use of the parent alcohol, generating the same allyloxyl radical than the hydroperoxide by photolysis of the corresponding nitrite formed with t-BuONO, also playing the role of the spin trap. It is suggested that the generation of these carbon radical species could play an important role for the binding of the hydroperoxide with skin proteins to form antigenic structures, the first step of the skin sensitization mechanism.

Cal K. (2007) “The type of carrier determines skin irritation caused by linalool & terpinen-4-ol.” *Int J Essen Oil Therapeutics* **1**, 1-3. Abstract. Linalool & terpinene-4-ol are terpenoids that are widely used in different products. Both of these

compounds easily penetrate specific skin layers and `can cause irritation reactions. The object of the present study was to evaluate the influence of two dermatological vehicles (hydrogel and oily solution) on potential skin irritation indicated by linalool and terpinene-4-ol in humans. The preparation containing terpenes were applied for 1 h onto the skin of ventral forearms. Irritation reactions were demonstrated by both terpenes applied in the hydrogel vehicle only. The effect is assumed to be directly related to the higher stratum corneum absorption of the terpenoid alcohols from the hydrophilic vehicle than from the oily solution. ***Cropwatch comments***: Synthetic ± linalool and terpinene-4-ol of (only) 97% purity from Fluka were used for the studies. No chemical analysis of the 3% impurities or peroxide value of the synthetics is given.

Cal K. & Kryzaniak M, (2006) "Stratum corneum absorption and retention of linalool and terpinene-4-ol applied as gel or oily solution in humans." *J. Dermatol. Sci* **42**, 265-267.

Cal K. (2006) "How does the type of vehicle influence the in vitro skin absorption and elimination kinetics of terpenes? *Arch Dermatol Res*. **297**(7), 311-5. Abstract. Terpenes are widely used in the topical dermal preparations, cosmetics and toiletries and also in the experimental dermopharmacy, as penetration enhancers. Terpenes do not need to penetrate into viable skin tissue and this event is not even desired. The aim of this study was to investigate skin absorption and elimination kinetics of two terpenes, namely linalool and terpinen-4-ol, incorporated in three different dermatological vehicles: oily solution, hydrogel and o/w emulsion. The preparations were applied onto the human skin in vitro, and after 1-4 h the content of terpenes in the stratum corneum layers and in the epidermis/dermis was determined using GC. Similarly, the amounts of terpenes in the skin were analysed during 4 h elimination process following 1 h absorption. The highest skin absorption was observed when terpenes were applied in hydrogel--their total content in the skin after 4 h was 385 and 705 microg/cm2 for linalool and terpinen-4-ol, respectively. After 1 h of the elimination process about 10-20% drop of the total content of both terpenes in the skin was noted for all formulations. The skin penetration of both terpenes from the vehicles is increasing in the following order: emulsion < oily solution < hydrogel, while the elimination phase is relatively slower for terpenes applied in hydrogel.

Cindy A. Ryan G. F. Gerberick, L. W. Cruse, Basketter D.A., Lea L., Blaikie L., Rebecca J., Dearman, E. V. Warbrick & Kimber I. (2000)." Activity of human contact allergens in the murine local lymph node assay." *Contact Dermatitis*, **43**,:95-102..

Coulson I.H. & Khan A.S.A (1999). "Facial 'pillow' dermatitis due to lavender oil allergy." *Contact Dermatitis* **41**(2), 111-111 **?**,

Hagvall L. (2009) *Formation of skin sensitizers from fragrance terpenes via oxidative activation routes: Chemical analysis, structure elucidation PhD Thesis University of Gothenberg.* Abstract. The work presented in this thesis emphasizes the importance of considering oxidative activation in the toxicity

assessment of fragrance chemicals Compounds without contact allergenic properties can be activated either via autoxidation in contact with air or via cutaneous metabolism to reactive products which can cause contact allergy. It is important to prevent sensitization as the immunological memory formed in the development of contact allergy persists throughout life. The investigation of compounds susceptible to oxidative activation, thereby forming sensitizing compounds is important in the work of prevention of contact allergy. The overall aim of this thesis was to investigate mechanisms of activation via autoxidation and metabolism of single fragrance compounds and essential oils, and to study the impact of this activation on the contact allergenic activity. The oxidative activation via autoxidation and cutaneous metabolism of the fragrance compounds geraniol and geranial was studied. It was shown that both compounds were susceptible to autoxidation, forming oxidation products with increased sensitizing capacity compared to the original compound. The oxidation products of geraniol were formed by two separate pathways, corresponding to autoxidation of each of the two double bonds in geraniol, respectively. Hydroperoxides, which previously have been identifed as the most important sensitizers in the oxidation mixtures of airexposed fragrance compounds could not be detected in air-exposed geranial.Instead, a sensitizing epoxide was detected. Geraniol and geranial were also activated metabolically. Many of the metabolites identitled were also present in the autoxidation mixtures. The autoxidation of lavender oil was studied in order to investigate if essential oils possess a natural protection against autoxidation. The results were compared to the results from the autoxidation studies of linalyl acetate and linalool, the main components of lavender oil. It was found that the autoxidation proceeded in the same way in both the pure samples and the lavender oil, and that sensitizing oxidation products were formed in both cases. The most important sensitizers formed were hydroperoxides of linalool and linalyl acetate. This thesis adds important information on routes of autoxidation as well as on the relationship between metabolic and air induced activation of non - or weakly sensitizing compounds to sensitizers. The results presented here indicate that other fragrance terpenes could be susceptible to oxidative activation via autoxidation or cutaneous metabolism. This should be considered in the risk assessment of fragrance chemicals.

Hagvall L., Sköld M., Bråred-Christensson J., Börje A. & Karlberg A.T. (2008) "Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure." *Contact Dermatitis*. **59**(3), 143-50. Abstract. BACKGROUND: Lavender oil is an essential oil frequently used as a fragrance ingredient and in traditional herbal medicine. We have previously studied the effect of air oxidation on the skin sensitizing potency of the monoterpenes linalyl acetate, linalool and beta-caryophyllene, the main constituents of lavender oil. OBJECTIVE: The aim of this study was to investigate if the autoxidation observed for the single synthetic terpenes, resulting in strong contact allergens, will take place also in lavender oil. METHODS: Lavender oil was exposed to air and the autoxidation was followed by chemical analysis. The sensitizing potency before and after air exposure was investigated in mice using the local lymph

node assay. Patients with patch test reactions to oxidized linalool were tested to investigate if air-exposed lavender oil could elicit dermatitis in these individuals. RESULTS: The terpenes oxidized in air-exposed lavender oil at the same rates as the pure compounds exposed to air, and the same oxidation products were identified. The sensitizing potency of lavender oil increased accordingly on air exposure. Patch testing showed positive reactions to air-exposed lavender oil and also to oxidized linalyl acetate in patients with contact allergy to oxidized linalool. CONCLUSION: This study shows that lavender oil lacks natural protection against autoxidation, and that air-exposed lavender oil can be an important source of exposure to allergenic hydroperoxides.

Hostynek J.J. & Malbach H.I. (2003) "Is there vevidence that linalool causes allergic contact dermatitis?." *Exogenous Dermatology* **2**, 223-9. Abstract. The fragrance material linalool has been cited as a moderately frequent cause of allergic contact dermatitis. The literature shows that when the underlying clinical and experimental data are analysed, a clear cause-effect relationship has infrequently or rarely been established. On the basis of the generally weak sensitizing potential of this substance coupled with its generally low exposure conditions, the prevalence of clinical cases would not be expected to be particularly high. This is not to say that linalool is a frequent inducer of type IV allergy in members of the public. However, it remains to be seen how often such allergy, once established, is responsible for any of the cases of allergic contact dermatitis commonly ascribed in the literature. Indeed in some cases, patch test conditions may not be optimal for differentiating between clinically relevant and irrelevant allergy to linalool.

Meesters R.J.W, Duisken M. & Hollender J. (2007). "Study on the cytochrome p450-mediated oxidative metabolism of the terpene alcohol linalool: Indication of biological epoxidation. *Xenobiotica* **37**(6):604-617.

Silvana W.A. (2004) "Linalool containing essential oils: new `safe-use proposal [monograph at http://www.soap.wire.com/2004/linaloolcontain.htm].

Sköld M, Hagvall L, Karlberg AT. (2008) "Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens." *Contact Dermatitis*. **58**(1):9-14. Abstract. BACKGROUND: Fragrances are among the most common causes of allergic contact dermatitis. We have in previous studies shown that linalool, present in lavender oil, autoxidizes on air exposure, forming allergenic oxidation products. Oxidized linalool was found to be a frequent cause of contact allergy in a patch test study on consecutive dermatitis patients. Linalyl acetate, the main component of lavender oil is commonly used as a fragrance chemical in scented products. Because of structural similarities, linalyl acetate should also be susceptible to oxidation on air exposure, forming similar oxidation products as linalool. OBJECTIVE: The aim of the present study was to investigate the autoxidation of linalyl acetate and the influence of oxidation on its sensitizing potency. METHODS: Analyses were performed using gas chromatography, nuclear magnetic resonance spectrometry and mass spectrometry. Sensitizing potencies of compounds were determined using the local lymph node assay

(LLNA) in mice. RESULTS: Analyses showed that the content of linalyl acetate decreased over time on air exposure and other compounds were formed. Hydroperoxides, an epoxide and an alcohol were identified as oxidation products from linalyl acetate. In the LLNA, linalyl acetate of high purity showed a weak sensitizing potency (EC3 25%). Autoxidation increased the sensitizing potency of linalyl acetate, and a 10 weeks oxidized sample gave an EC3 value of 3.6%. As for linalool, the hydroperoxides were shown to be the oxidation products with the highest sensitizing potency. CONCLUSION: It is concluded that autoxidation of the weakly allergenic linalyl acetate leads to formation of allergenic oxidation products.

Skold M., Borje A., Harambasic E., &. Karlberg A.T. (2004). "Contact allergens formed on air exposure of linalool. identification and quantification of primary and secondary oxidation products and the effect on skin sensitization." *Chem Res Toxicol*, **17**(12):1697-705. Abstract. Linalool (3,7-dimethyl-1,6-octadien-3-ol) is an important fragrance chemical, frequently used in scented products because of its fresh, flowery odor. Linalool is an unsaturated hydrocarbon and is therefore susceptible to oxidation in the presence of air. The primary oxidation products, that is, hydroperoxides, formed in the autoxidation process, are reactive compounds that can be suspected to act as sensitizers. In the present investigation, we studied the autoxidation of linalool with emphasis on the formation of hydroperoxides. The oxidation products were isolated using flash chromatography and preparative HPLC and were identified with NMR and GC/MS, using synthesized reference compounds. Two hydroperoxides and several different secondary oxidation products were identified, among which some contain structural features that make them potential allergens. The amounts of linalool and the major oxidation products were quantified over time, using GC and an HPLC-method, suitable for the analysis of thermolabile primary oxidation products. The hydroperoxide 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol was found to be present in 15% in an oxidized sample. The local lymph node assay (LLNA) was used to investigate the sensitizing potential of pure linalool, two samples of air-exposed linalool, and oxidation products of linalool (an alpha,beta-unsaturated aldehyde, a mixture of two hydroperoxides, and an alcohol). Pure linalool showed no sensitizing potential. The air-exposed samples of linalool produced clearly positive responses, and the hydroperoxides were the strongest allergens of the tested oxidation products. The study demonstrates the importance of autoxidation on the sensitizing potential of linalool. We also conclude that the sensitizing potential differs with the composition of the oxidation mixture and thus with the air exposure time.

Skold M., Borje A., Matura M., Karlberg A.T. (2002) "Sensitisation studies on fragrance chemical linalool with respect to auto-oxidation." *Contact Dermatitis* **26** Suppl 20.

Skold M., Borje A., Matura M., Karlberg A.T. (2002) "Studies on the autoxidation & sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide." *Contact Dermatitis* **46**, 267-272. Abstract. Fragrances are among

the most common causes of allergic contact dermatitis. The two monoterpenes linalool and d-limonene are the most frequently incorporated fragrance chemicals in scented products. Previous studies on d-limonene show that this monoterpene oxidizes on air exposure (autoxidation) and that allergenic oxidation products are formed. Due to structural similarities, linalool might also form allergenic oxidation products on air exposure. The aim of the present study was to study the autoxidation of linalool and to investigate the sensitizing potential of linalool before and after air exposure. Linalool was oxidized for 10 weeks and gas chromatographic analyses showed that the content of linalool decreased to about 80%. The chromatograms revealed the formation of other compounds during oxidation. One of the major oxidation products was isolated and identified as 7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol. This substance is, to the best of our knowledge, described for the first time. In sensitization studies in guinea pigs, linalool of high purity gave no reactions, while linalool that had been oxidized for 10 weeks sensitized the animals. It is concluded that autoxidation of linalool is essential for its sensitizing potential.

Skold M., Borje A., Matura M. & Karlberg A.-T. (2002) "Air exposure of linalool, the most frequently used fragrance material creates allergenic oxidation products. Testing with the oxidized limonene mixture and its allergenic fractions." Presented at the *17th Meeting of the European Research Group on Experimental Contact Dermatitis, Strasbourg, France Jan 18-20, 2002*

Varma S., Blackford S., Statham B.N., Blackwell A. (2000). "Combined contact allergy to tea tree oil and lavender oil complicating chronic vulvovaginitis." *Contact Dermatitis*, **42**(5):309-310. .

Lippia multiflora

Oladimeji F.A. & Orafidiya L.O. (2007) "A` study on the autoxidation profile of leaf essential oil of *Lippia multiflora* Moldenke under different storage conditions." Int *J Essential Oil Therapeutics* **1**, 39-44. Abstract. The autoxidation profile of lippia oil, an essential oil hydrodistilled from the leaves of *Lippia multiflora* Moldenke was studied under varying storage conditions. Freshly distilled oil was stored half or fully filled in brown/colourless bottles in a refrigerator (6º ± 1°C.) or on the shelf (28° ± 1°C.) with or without butylated hydroxyanisole (BHA) for 12 months. There were significant changes ($P<0.05$) in refractive indices and pH values of oil samples stored on the shelf, with pH values (≤ 3.83) lower than that of freshly distilled lippia oil (4.75). An eight-fold increase in oxidation level, expressed as Active Oxygen Number (AON) of oil stored in half-filled bottles on the shelf compared with that stored in a refrigerator was observed. Autoxidation of lippia oil samples followed first-order kinetics ($r2 \geq 0.950$). A mean oxidation index of 7.04 ± 0.61 was derived and used in calculating induction periods of the oil samples. The induction periods calculated for lippia oil samples stored in the refrigerator with 0.1% BHA, without BHA, and those stored on the shelf with 0.1% BHA were 29., 19.41 & 15.19 months, respectively, compared with 6.60 months obtained for oil sample without BHA, stored in half-filled brown bottles on the shelf. The mean stability indices calculated for refrigeration, and 0.1% BHA were

2.452 ± 0.026 and 1.774 ± 0.206 respectively. The results of the study suggest that storage in a refrigerator could provide protection against autoxidation of lippia oil. In the absence of refrigerating facilities, inclusion of antioxidant (0.1% BHA) in lippia oil would prevent a build-up of hydroperoxides and other products on aging.

Oladimeji F.A. & Orafidiya L.O. Okeke I.N. & Dagne N. (2001). “Effect of autoxidation on the composition and antimicrobial activity of essential oil of *Lippia multiflora*.” *Pharm. Pharmocol. Lett*. **2**, 64-7.

Mint oils.

Ito M., Sagawa S. Abe K & Onogaki T. (1969) "Isolation of menthofurolactone from *Mentha arvensis* and solvent effect of oil components on the formation. Paper presented at the *12th Symposium on Terpenes,Essential Oils & Aromatics Hamamatsu, Oct 1968.* Abstract. Menthofurolactoine was isolated from Mentha arvensis by alkali extraction. The solvent effects oif the oil components on autoxidation of menthofuran into menthofurolactone was measured.

Frérot E., Bagnoud A. & Vuilleumier C. (2002) "Menthofurolactone: a new p-menthane lactone in *Mentha piperita* L.: analysis, synthesis and olfactory properties." *Flav. & Frag J.* **17**(3), 216-226. Abstract. 3,6-Dimethyl-4,5,6,7-tetrahydro-benzo[b]-furan-2(3H)-one (= menthofurolactone), first reported as a by-product in the oxidation of menthofuran and hitherto unknown in nature, is shown to be naturally occurring in *Mentha piperita* L. essential oil. In addition to a description of its synthesis and organoleptic properties, the key role of menthofurolactone in the flavour profile of peppermint oil is demonstrated by mint oil analysis and olfactory studies.

Myrrh oil.

Auffray, B. (2007) "Protection against singlet oxygen, the main actor of sebum squalene peroxidation during sun exposure, using *Commiphora myrrha* essential oil." *Int J Cosmetic Science* **29**(1), 23-29. Abstract. Squalene is a component of sebum. Both are directly exposed to the external environment and play a key role in skin physiology. They are particularly prone to photo oxidation during sun exposure. We studied the impact of two types of antioxidant on sebum squalene peroxidation by UV irradiation. The first type is free radical scavenger (Butyl hydroxyl toluene and an olive extract rich in hydroxytyrosol). The second type is the essential oil of *Commipora myrrha*, a singlet oxygen quencher. These properties were confirmed using the 2,2-diphenyl-1-picrylhydrazyl test for antiradical capacity [Yoshida *et al*. (1989) *Chem. Pharm. Bull.,* **37**, 1919; Buenger *et al*. (2006) *Int. J. Cosmet. Sci*., **28**, 135] and 1,3-diphenylisobenzofuran test for the capacity to quench singlet oxygen [Kochewar and Redmond (2000) *Meth. Enzymol*., **28**, 319; Racine and Auffray (2005) *Fitoterapia*, **76**, 316]. Furthermore, we have extended an ex vivo method to classify the efficacy of cosmetics to protect squalene by collecting sebum in vivo and irradiating it in a controlled way. The squalene monohydroperoxide formation is monitored by high performance liquid chromatography. This methods allows us to compare the efficiency of the three antioxidants at 0.6% in a cosmetic

formulation to protect squalene from photo oxidation. Our results clearly show that essential oil of *Commiphora myrrha* provides the best protection against squalene peroxidation. These results demonstrate that squalene peroxidation during solar exposure is mainly because of singlet oxygen and not due to free radical attack. This suggests that sun care cosmetics should make use not only of free radical scavengers but also of singlet oxygen quenchers.

Oregano oil

Kulisic T., Radonic A., Katalinic V. & Milos M. (2003) "Use of different methods for testing antioxidative activity of oregano essential oil." *Food Chem* **85**(4), 633-640. Abstract. The antioxidant properties of the essential oil from oregano in relation to its chemical composition were examined. The antioxidant activity was investigated with three different methods: the β-carotene bleaching (BCB) test, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the thiobarbituric acid reactive species (TBARS) assay. It was found that the total essential oil, its fraction as well as its pure constituents have a significant antioxidant effect when tested by each method, respectively. Generally the antioxidant activity of the oregano essential oil is less effective than the ascorbic acid, but comparable with the α-tocopherol and the synthetic antioxidant butylated hydroxytoluene (BHT). The synergy among minor oxygen containing compounds was suggested as possible factor, which influenced the antioxidant power of the oregano essential oil. The antioxidant concentrations influenced its antioxidant power, too.

Lagouri V., Blekas G., Tsimidou M. Kokkini S. & Boskou D. (1993) "Composition and antioxidant activity of essential oils from Oregano plants grown wild in Greece." *Zeitschrift für Lebensmitteluntersuchung und Forschung A*. **197**(1), 20-23. Abstract. In this study, four samples of essential oils obtained from plant species with a wide distribution in Greece and economic importance for the world-wide spice market and also carvacrol and thymol were tested for their possible antioxidant activity. The four plant species were *Origanum vulgare* subsp. *hirtum*, *O. onites*, *Coridothymus capitatus* and *Satureja thymbra*. The essential oils were chemically characterised by gas chromatography-ion trap detection. All the essential oils examined and also carvacrol and thymol were found to have antioxidant activity when tested on TLC plates and by measuring peroxide values of lard stored at 35° C. The results indicate that the antioxidant effect may be related to the presence of carvacrol and thymol in essential oils. ***Cropwatch comments: The authors refer to the work of Farag et al. and maintain that there is a relationship between the inhibition of hydroperoxide formation and the presence of thymol & eugenol in some essential oils.***

Pine oil

Tammela P., Nygren M., Laakso I., Hopia A., Vuorela H. & Hiltunen R. (2003) "Volatile Compound analysis of ageing *Pinus sylvestris* L. (Scots pine) seeds." *Flav & Frag J*. **8**(4), 290-295. Abstract. The volatile compound profiles of 11 Scots pine (*Pinus sylvestris* L.) seed lots were determined by solid phase

microextraction combined with GC-MS analysis. Typical monoterpene constituents of pine volatile oils were identified, and total monoterpene content of these seeds was measured semi-quantitatively as peak area. Considerable variation in monoterpene composition was found between seeds of different origin, especially in two major constituents, 3-carene and -pinene. The relative concentrations of these compounds varied between 0-67 and 16-52%, respectively. Comparison of total monoterpene amounts showed that reduced monoterpene content was typical for aged, poorly germinating seeds. However, high germinability was not always associated with a high monoterpene content. Therefore, no clear dependency between monoterpene content and seed ageing could be established based on this data. The possible protective role of monoterpenes in pine seed ageing during long-term storage is discussed. In addition, a drastic increase in volatile lipid oxidation products was found in the oldest seed lots. In 20 year-old seeds, the percentage of hexanal, an oxidation product of fatty acids, was 3% of the total volatiles, whereas in 31 year-old seeds it was 17%. No hexanal or other lipid oxidation products were detected in younger seeds.

Pinene, *alpha-*

Ancel J.E., Maksimchuk N.V., Simakova I.L. & Semikolenov V.A. (2004) "Kinetic peculiarities of α-pinene oxidation by molecular oxygen" *Applied Catalysis A*. **272**(1-2), 109-114. Abstract. Kinetic peculiarities of α-pinene liquid-phase oxidation by molecular oxygen were studied (in a temperature range of 343–393 K and at oxygen pressure within 0.5–6 bar range). The process proceeds selectively at a low α-pinene conversion and the main products are verbenyl-hydroperoxide and α-pinene epoxide. The products ratio depends slightly on temperature, oxygen pressure and α-pinene conversion. The kinetic equation for the reaction rate was suggested.

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Singh H.P., Batish D.R., Kaur S., Arora K. & Kohli R.K.. (2006) "alpha-Pinene inhibits growth and induces oxidative stress in roots." *Ann Bot (Lond).* **98**(6),1261-9. Abstract. BACKGROUND AND AIMS: Determining the mode of action of allelochemicals is one of the challenging aspects in allelopathic studies. Recently, allelochemicals have been proposed to cause oxidative stress in target tissue and induce an antioxidant mechanism. alpha-Pinene, one of the common monoterpenoids emitted from several aromatic plants including forest trees, is known for its growth-inhibitory activity. However, its mechanism of action remains unexplored. The aim of the present study was to determine the inhibitory effect of alpha-pinene on root growth and generation of reactive oxygen species, as indicators of oxidative stress and changes in activities of antioxidant enzymes. METHODS: Effects of alpha-pinene on early root growth were studied in five test species, *Cassia occidentalis*, *Amaranthus viridis*, *Triticum aestivum*, *Pisum sativum* and *Cicer arietinum*. Electrolyte leakage, lipid peroxidation, hydrogen peroxide generation, proline accumulation, and activities of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT) and glutathione reductase (GR) were studied in roots of *C. occidentalis*. KEY RESULTS: alpha-Pinene inhibited the radicle growth of all

the test species. Exposure of *C. occidentalis* roots to alpha-pinene enhanced solute leakage, and increased levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. Activities of the antioxidant enzymes SOD, CAT, GPX, APX and GR were significantly elevated, thereby indicating the enhanced generation of reactive oxygen species (ROS) upon alpha-pinene exposure. Increased levels of scavenging enzymes indicates their induction as a secondary defence mechanism in response to alpha-pinene. CONCLUSIONS: It is concluded that alpha-pinene inhibits early root growth and causes oxidative damage in root tissue through enhanced generation of ROS, as indicated by increased lipid peroxidation, disruption of membrane integrity and elevated antioxidant enzyme levels.

Santalene, *α-*

Ngo K-S & Brown G.D. (2000) "Autoxidation of α-santalene" *Journal of Chemical Research* **2000**(2),68-70(3). Abstract. Fifteen compounds (2 - 11) have been isolated from the spontaneous slow autoxidation of the tri-substituted double bond in the side-chain of the tricyclic sesquiterpene α-santalene; most of these compounds have also been reported as natural products.

Squalene

Auffray, B. (2007) "Protection against singlet oxygen, the main actor of sebum squalene peroxidation during sun exposure, using *Commiphora myrrha* essential oil." *Int J Cosmetic Science* **29**(1), 23-29. Abstract. Squalene is a component of sebum. Both are directly exposed to the external environment and play a key role in skin physiology. They are particularly prone to photo oxidation during sun exposure. We studied the impact of two types of antioxidant on sebum squalene peroxidation by UV irradiation. The first type is free radical scavenger (Butyl hydroxyl toluene and an olive extract rich in hydroxytyrosol). The second type is the essential oil of *Commipora myrrha*, a singlet oxygen quencher. These properties were confirmed using the 2,2-diphenyl-1-picrylhydrazyl test for antiradical capacity [Yoshida *et al*. (1989) *Chem. Pharm. Bull.,* **37**, 1919; Buenger *et al*. (2006) *Int. J. Cosmet. Sci*., **28**, 135] and 1,3-diphenylisobenzofuran test for the capacity to quench singlet oxygen [Kochewar and Redmond (2000) *Meth. Enzymol*., **28**, 319; Racine and Auffray (2005) *Fitoterapia*, **76**, 316]. Furthermore, we have extended an ex vivo method to classify the efficacy of cosmetics to protect squalene by collecting sebum in vivo and irradiating it in a controlled way. The squalene monohydroperoxide formation is monitored by high performance liquid chromatography. This methods allows us to compare the efficiency of the three antioxidants at 0.6% in a cosmetic formulation to protect squalene from photo oxidation. Our results clearly show that essential oil of *Commiphora myrrha* provides the best protection against squalene peroxidation. These results demonstrate that squalene peroxidation during solar exposure is mainly because of singlet oxygen and not due to free radical attack. This suggests that sun care cosmetics should make use not only of free radical scavengers but also of singlet oxygen quenchers.

Tea tree oil (*separate Cropwatch Tea Tree Oil Bibliography in preparation*).

Ernst E. & Huntley A. (2000) "Tea Tree Oil: A Systematic Review of Randomized Clinical Trials." *Research in Complemetary Medicine* **7**(1), 17-20. Abstract. Aim: Tea tree oil (TTO) is immensely popular for various topical applications. In vitro studies have repeatedly demonstrated that it has antibiotic activity. This article is an attempt to systematically review the evidence from randomised clinical trials for or against effectiveness of external TTO in dermatological conditions. Methods:Six electronic databases were searched. Methodological quality was assessed by Jadad score. Data were extracted and validated in a standardised fashion by two independent reviewers. Results:Only 4 trials were located. They suggest that TTO may be effective as a treatment of acne and fungal infections. The evidence is promising but by no means compelling. The adverse effects of TTO are usually mild and transient. They mainly consist of allergic reactions. Conclusions:It is concluded that, so far, there is no compelling evidence to show that TTO is efficacious in any dermatological condition. However, in view of promising findings, TTO deserves to be investigated more closely

Fritz T.M., Burg G. & Krasovec M. (2001) "[Allergic contact dermatitis to cosmetics containing *Melaleuca alternifolia* (tea tree oil)] *Ann Dermatol Venereol.* **128**(2), 123-6. Abstract. INTRODUCTION: *Melaleuca alternifolia* is a coniferous tree found in tropical regions, the needles contain an essential oil that is used in medical and cosmetic products. The essential oil contains turpentines (limonene, alpha-pinene, phellandrene) that are potentially allergenic.PATIENTS AND METHODS: In 1997, 1216 patients were patch tested in our dermatoligic unit. Fourteen of them tested because of eczema used products containing tea tree oil. The patients used creams, hair products and essential oils containing *Melaleuca alternifolia* for cosmetic reasons and to treat skin affections. They were patch tested for a standard panel of allergens, topical emulgators, perfumes, plants, topical medications, metal, gloves, topical disinfectants and preservatives, dental products and rubber derivatives. Products containing *Melaleuca alternifolia* were tested concentrated or diluted.RESULTS: We report on 7 cases of patients with an allergic contact dermatitis due to tea tree oil. Two of them also exhibited from a delayed type IV hypersensitivity towards fragrance-mix or colophony suggesting the possibility of cross reaction or an allergic group reaction caused by contamination of the colophony with the volatile fraction of turpentines.DISCUSSION: The allergic potential of low concentrations of *Melaleuca alternifolia* is presumed to be low on healthy skin. Photoaged *Melaleuca alternifolia* must be considered to be a stronger sensitizer.

de Groot AC (1996) “Airborne allergic contact dermatitis from tea tree oil.” *Contact Dermatitis* **35**, 304-5

de Groot AC & Weyland J.W. (1992) “Systemic contact dermatitis from tea tree oil.”. *Contact Dermatitis* **27**, 279-80

Hammer K.A., Carson C.F., Riley T.V. & Nielsen J.B. (2006) "A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil." *Food Chem Toxicol.* **44**(5), 616-25. Abstract. The essential oil of Melaleuca alternifolia, also known as tea tree or melaleuca oil, is widely available and has been investigated as an alternative

antimicrobial, anti-inflammatory and anti-cancer agent. While these properties are increasingly well characterised, relatively limited data are available on the safety and toxicity of the oil. Anecdotal evidence from almost 80 years of use suggests that the topical use of the oil is relatively safe, and that adverse events are minor, self-limiting and occasional. Published data indicate that TTO is toxic if ingested in higher doses and can also cause skin irritation at higher concentrations. Allergic reactions to TTO occur in predisposed individuals and may be due to the various oxidation products that are formed by exposure of the oil to light and/or air. Adverse reactions may be minimised by avoiding ingestion, applying only diluted oil topically and using oil that has been stored correctly. Data from individual components suggest that TTO has the potential to be developmentally toxic if ingested at higher doses, however, TTO and its components are not genotoxic. The limited ecotoxicity data available indicate that TTO is toxic to some insect species but more studies are required.

Harkenthal M., Reichling J., Geiss H.K. & Saller R (1998) “Oxidationsprodukte als mögliche Ursache von Kontaktdermatitiden.”. *Pharmazeut Z* , **47,** 4092.

Harkenthal M., Hausen B.M. & Reichling J (2000) “1,2,4-Trihydroxy menthane, a contact allergen from oxidized Australian tea tree oil. *Pharmazie* **55**, 153-4.

Hausen B.M. (2004) “Evaluation of the main contact allergens in oxidised tea tree oil.” *Dermatitis* **15**, 213-4. Abstract. BACKGROUND: Patients using tea tree oil (TTO) topically may become sensitized to this natural remedy. More than 30 cases have been documented in the literature since 1991. OBJECTIVE: Freshly distilled, as well as oxidized TTO, some fractions, and single constituents were used for experimental sensitization in guinea pigs. TTO was stored on a window sill to study the influence of light, oxygen, and warmth. The oxidized oil and different fractions were devoted to experimental sensitization in guinea pigs to determine their sensitizing potency. Fifteen constituents were patch tested in TTO-sensitive patients to find how many may play a role as contact allergens. METHODS: Guinea pigs were sensitized by a modified FCA-method (Freund's complete adjuvant) with freshly distilled TTO, oxidized TTO, the monoterpene and sesquiterpene fraction, and 1, 8-cineole. TTO-sensitive patients were tested with 15 typical constituents and degradation products. Gas chromatographic analysis was used to detect degradation products of the deteriorated TTO. RESULTS: Fresh TTO was revealed to be a very weak sensitizing material whereas oxidized TTO was 3 times stronger. The monoterpene fraction showed to be a stronger sensitizer than the sesquiterpene fraction. All 11 patients reacted mostly with a ++-plus or even a -plus reaction to alpha-terpinene, terpinolene and ascaridol. alpha-phellandrene became positive in four patients, myrcene in only two. Gas chromatographic analyses showed that the formation of peroxides increased within 4 days from less than 50 to more than 500 ppm. Peroxides, epoxides and endoperoxides were formed. Deterioration products of alpha-terpinene were found to be mainly p-cymene, ascaridol, isoascaridol, a ketoperoxide, and colorless crystals that likely were 1,2,4-trihydroxy menthane. The p-cymene content increased dramatically from 2% to 11.5%. alpha- and

gamma-terpinene, as well as terpinolene, were reduced to one half of their former concentration. Ascaridol and isoascaridol have never before been found in TTO. CONCLUSION: Tea tree oil kept in open and closed bottles or other containers undergoes photooxidation within a few days to several months, leading to the creation of degradation products that are moderate to strong sensitizers. Peroxides, epoxides and endoperoxides, like ascaridol and 1,2,4-trihydroxy menthane, are formed. These must be considered responsible for the development of allergic contact dermatitis seen in individuals treating themselves with the oil. A test series with 15 characteristic constituents is recommended for patch testing.

Hausen BM, Reichling J & Harkenthal M (1999) Degradation products of monoterpenes are the sensitizing agents in tea tree oil. *Am J Contact Dermat* **10**:68-77.

Holschbach M., Breuer S., Wilbers L., Merk H.F., Neugebauer M., Hollender J.& Blömeke B. (2008) "Characterisation of dendritic cell responses to ascaridol, an autoxidation product of tea tree oil." *Contact Dermatitis* **50**(3), 170-171. Abstract. A large number of monoterpenes and their degradation products are likely skin sensitizing agents (Hausen et al., 1999). Terpenes are very common e.g., as constituents of cosmetics, food and other daily used products. We investigated responses to the endoperoxide 1,4 - epidioxy - 2 - p - menthen (ascaridol), an autoxidation product of tea tree oil, using monocyte derived dendritic cells (MDDC). Therefore, we isolated peripheral blood mononuclear cells (PBMC) from 9 healthy donors by the standard Ficoll - Paque gradient centrifugation. Monocytes were isolated by adherence and incubated in media (RPMI 1640) containing GM - CSF (800 units/ml), IL - 4 (1000 units/ml) and 10% autologous serum. The immature MDDC (day 6) were characterized by flow cytometry (CD1a+, CD14–, CD40, CD45, CD80, CD83, CD86, HLA - DR and CCR - 7) and incubated with various concentrations of ascaridol (1–70 µg/ml). After one hour incubation time LPS was added (1 µg/ml) for 23 h. Cell culture supernatants were collected after 24 h for cytokine analysis. IL - 12p40, IL - 12p70 and prostaglandin E2 were measured by ELISA, TNF - alpha and IL - 2 were measured by flow cytometry (FACS). Methods of the quantification of steady state mRNA levels were established for IL - 12 and CCR7 (real - time RT - PCR). Ascaridol enhanced significantly IL - 12p70 production (120% up to 396%) by MDDC as well as mRNA levels for IL - 12 and CCR7. Moreover, we detected a distinct increase of TNF - alpha (110% up to 146%) secretion, IL - 2 (135%) and PGE2 (102% up to 155%).
Totally, these results suggest that ascaridol may be a potent modulator of maturation and antigen presenting function of dendritic cells, and we performing further experiments to verify this hypothesis.

Kim H.J., Chen F., Wu C., Wang X., Chung H.Y. & Jin Z (2004) "Evaluation of antioxidant activity of Australian tea tree (*Melaleuca alternifolia*) oil and its components." *J Agric Food Chem.* **52**(10), 2849-54. Abstract. Antioxidant activity of Australian tea tree (Melaleuca alternifolia) oil (TTO) was determined using two

different assays. In the 2,2-diphenyl-1-picrylhydrazyl assay, 10 microL/mL crude TTO in methanol had approximately 80% free radical scavenging activity, and in the hexanal/hexanoic acid assay, 200 microL/mL crude TTO exhibited 60% inhibitory activity against the oxidation of hexanal to hexanoic acid over 30 days. These results were equivalent to the antioxidant activities of 30 mM butylated hydroxytoluene in both tests at the same experimental conditions. This indicated that the TTO could be a good alternative antioxidant. Inherent antioxidants, i.e., alpha-terpinene, alpha-terpinolene, and gamma-terpinene, in the crude TTO were separated and identified chromatographically using silica gel open chromatography, C(18)-high-pressure liquid chromatography, and gas chromatography-mass spectrometry. Their antioxidant activities decreased in the following order in both assays: alpha-terpinene > alpha-terpinolene > gamma-terpinene.

Rubel D.M., Freeman S., Southwell I/A. “Tea tree allergy: what is the offending agent? Report of three cases of tea tree allergy and reviews of the literature.” *Australas. J. Dermatol.* **59**, 244-7. Abstract. Tea tree oil is currently enjoying popularity as a cureall for a variety of skin conditions, from infections to psoriasis, and many household and personal products containing Melaleuca oil are available. However, despite its chemical complexities and enthusiastic use, there have been only a few reports of allergic reactions lo lea tree oil. At the Skin and Cancer Foundation (Sydney, NSW, Australia), three of 28 normal volunteers tested strongly positive to patch testing with tea tree oil. Following farther patch testing with tea tree oil constituents, all three patients reacted strongly to two preparations containing sesquiterpenoid fractions of the oil. Because patients often neglect to mention that they have used "natural" remedies, it is important that physicians are aware of the potential adverse effects of these products. Furthermore, identification of the allergenic ingredients in lea tree oil may assist the growing industry to produce safer products.

Rutherford T, Nixon R, Tam M & Tate B. (2007) "Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years." *Australas J Dermatol.* **48**(2):83-7. Abstract. Tea tree oil use is increasing, with considerable interest in it being a 'natural' antimicrobial. It is found in many commercially available skin and hair care products in Australia. We retrospectively reviewed our patch test data at the Skin and Cancer Foundation Victoria over a 4.5-year period and identified 41 cases of positive reactions to oxidized tea tree oil of 2320 people patch-tested, giving a prevalence of 1.8%. The tea tree oil reaction was deemed relevant to the presenting dermatitis in 17 of 41 (41%) patients. Of those with positive reactions, 27 of 41 (66%) recalled prior use of tea tree oil and eight of 41 (20%) specified prior application of neat (100%) tea tree oil. Tea tree oil allergic contact dermatitis is under-reported in the literature but is sufficiently common in Australia to warrant inclusion of tea tree oil, at a concentration of 10% in petrolatum, in standard patch-test series. Given tea tree oil from freshly opened tea tree oil products elicits no or weak reactions, oxidized tea tree oil should be used for patch testing.

Varma S., Blackford S., Statham B.N., Blackwell A. (2000). “Combined contact allergy to tea tree oil and lavender oil complicating chronic vulvovaginitis.” *Contact Dermatitis*, **42**(5):309-310. .

Velen NK, Rosner K, Skovgaard GL. (2004) “Is tea tree oil an important contact allergen?” *Contact Dermatitis* **50**, 378-9.

Terpinene, γ-

Foti MC, Ingold KU. (2003) "Mechanism of inhibition of lipid peroxidation by gamma-terpinene, an unusual and potentially useful hydrocarbon antioxidant. *J Agric Food Chem.* **51**(9), 2758-65. Abstract. gamma-Terpinene (TH), a monoterpene hydrocarbon present in essential oils, retards the peroxidation of linoleic acid (LH). The peroxidation of TH has been shown to yield p-cymene as the only organic product in a chain reaction in which the chain carrier is the hydroperoxyl radical, HOO(.). The peroxidation of LH is well-known to be a chain reaction in which the chains are carried by linoleylperoxyl radicals, LOO., and the products are linoleyl hydroperoxides. The retardation of LH peroxidation by TH has been found to be due to rapid chain termination via a very fast cross-reaction between HOO. and LOO. radicals. This antioxidant mechanism is completely different from the mechanism of antioxidant action of vitamin E. Since vitamin E becomes a prooxidant at high concentrations, the addition of essential oils containing TH to edible lipids may provide an alternative or supplementary strategy for obtaining large increases in their oxidative stability and shelf life, something that cannot be achieved by simply adding more and more vitamin E.

Thyme oil.

Simandi, Bela (2001) “Antioxidant activity of pilot-plant alcoholic and supercritical carbon dioxide extracts of thyme.” *European Journal of Lipid Science and Technology* **103**(6).

Youdim,-K.A.; Deans,-S.G.; Finlayson,-H.J. (2002) "The antioxidant properties of thyme (*Thymus zygis* L.) essential oil: an inhibitor of lipid peroxidation and a free radical scavenger." *J Essn Oil Research*. **14**(3), 210-5. Abstract. Antioxidants minimize the oxidation of lipid components in cell membranes by scavenging free radicals. However, imbalance between free radical production and removal tends to increase with age causing progressive damage. For the food industry it is of considerable interest to delay the autoxidation of food lipids, which cause the reduction in food quality, affecting color, taste, nutritive value, and functionality. A general orientation toward the use of natural compounds has stimulated research into the potential use of aromatic and medicinal plants as possible antioxidant replacements. This study characterized the antioxidant and pro-oxidant properties of thyme oil and a number of its components. The major components identified in thyme oil were found to inhibit ferric-ion-stimulated lipid peroxidation of rat brain homogenates, although none was as effective as the whole oil. The order of antioxidant activity was; thyme oil greater than thymol greater than carvacrol greater than gamma-terpinene greater than myrcene greater than linalool greater than p-cymene greater than limonene greater than 1,8-cineole greater than alpha-pinene. Both thyme oil and thymol were also found to inhibit

tert-butyl-hydroperoxide-stimulated peroxidation and INT reduction by superoxide radicals generated by the xanthine-xanthine oxidase system. Of these compounds tested only p-cymene, 1,8-cineole and myrcene were found to exhibit pro-oxidant activity, albeit to a very small extent. Overall, the data suggest that thyme oil possesses useful antioxidant properties that may be utilized in the food industry and as a dietary supplement.

Turpentine

Treudler R, Richter G, Geier J, Schnuch A, Orfanos CE, Tebbe B. (2000) "Increase in sensitization to oil of turpentine: recent data from a Multicenter Study on 45,005 patients from the German-Austrian Information Network of Departments of Dermatology (IVDK)". *Contact Dermatitis* **42**, 68-73

Vegetable oils.

Campo P, Zhao Y, Suidan MT, Venosa AD, Sorial GA.(2007) "Biodegradation kinetics and toxicity of vegetable oil triacylglycerols under aerobic conditions." *Chemosphere* **68**(11), 2054-62. Abstract. The aerobic biodegradation of five triacylglycerols (TAGs), three liquids [triolein (OOO), trilinolein (LLL), and trilinolenin (LnLnLn)] and two solids [tripalmitin (PPP) and tristearin (SSS)] was studied in water. Respirometry tests were designed and conducted to determine the biochemical oxygen demand (BOD) parameters of the compounds. In the case of the solid lipids, the degradation process was limited by their extremely non-polar nature. When added to water, PPP and SSS formed irregular clumps or gumballs, not a fine and uniform suspension required for the lipase activity. After 30 days, appreciable mineralization was not achieved; therefore, first-order biodegradation coefficients could not be determined. The bioavailability of the liquid TAGs was restricted due to the presence of double bonds in the fatty acids (FAs). An autoxidation process occurred in the allylic chains, resulting in the production of hydroperoxides. These compounds polymerized and became non-biodegradable. Nevertheless, the non-oxidized fractions were readily mineralized, and BOD rate constants were estimated by non-linear regression: LLL (k=0.0061h(-1)) and LnLnLn (k=0.0071h(-1)) were degraded more rapidly than OOO (k=0.0025h(-1)). Lipids strongly partitioned to the biomass and, therefore, Microtox toxicity was not observed in the water column. However, EC(50) values (<15% sample volume) were measured in the solid phase.

Farag R.S., Badei A.Z.M.A., Hewedi F.M. & El-Baroty G.S.A. (2007) "Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media." *Journal of the American Oil Chemists' Society* **66**(6), 729-799. Abstract. Some spice essential oils (caraway, clove, cumin, rosemary, sage and thyme) and their major constituents were added to emulsified linoleic acid in aqueous media to examine their antioxidant activity. The methods used for measuring linoleic acid oxidation were coupled oxidation ofβ-carotene, conjugated diene formation and thiobarbituric acid test. The essential oils under study possess an antioxidant effect and this phenomenon was increased by increasing their concentration. Generally, the effectiveness of the various essential oils on linoleic acid oxidation was in the following descending order: caraway

>sage>cumin>rosemary>thyme>clove. It appears that there was a relationship between the antioxidant effect and the chemical composition of the oils.

Isnardy B., Wagner K.H. & Elmadfa I. (2003) "Effects of alpha-, gamma-, and delta-tocopherols on the autoxidation of purified rapeseed oil triacylglycerols in a system containing low oxygen." *J Agric Food Chem*. **51**(26), 7775-80. Abstract. Controversial data on the antioxidant effects of tocopherols have already been shown in different test systems, yet delta-tocopherol was hardly considered. This study was designed to assess the effects and degradation of alpha-, gamma-, and delta-tocopherol in four concentrations from between 0.01 and 0.25% on the oxidation of purified rapeseed oil trigacylglycerols (RO-TAG) at 40 degrees C in the dark in a low oxygen containing system for 11 weeks. Oxidation experiments were performed weekly by assessing primary (peroxide value, PV; conjugated dienes, CD) and secondary (p-anisidine reactive products, p-AV; hexanal) oxidation products, the degree of unsaturation with the iodine value (IV), and the stability of tocopherols. Test approaches were performed with and without the addition of 0.01% alpha,alpha'-azoisobutyronitrile (AIBN), which is a known radical initiator. alpha- and gamma-Tocopherols increased the rate of lipid oxidation, which was more pronounced in the presence of AIBN. Only the lowest amount of 0.01% gamma-tocopherol was comparable to the control sample in the test without AIBN. The most effective was shown to be delta-tocopherol, which did not elevate lipid oxidation except the PV in the AIBN test, but they did not delay it either. delta-Tocopherol was the most stable followed by gamma- and alpha-tocopherol. For alpha- and gamma-tocopherol, but not for delta-tocopherol, strong correlations were found between the tocopherol degradation and the extent of oxidation. Results suggest that (i). at concentrations higher than 0.05%, tocopherols are less efficient and turn their mode of action or participate in side reactions in RO-TAG and (ii). delta-tocopherol was shown to be the most stable and effective under these low oxygen conditions.

Miraliakbari H, Shahidi F. (2008) "Oxidative stability of tree nut oils." *J Agric Food Chem*. **56**(12), 4751-9. Abstract. The oxidative stability of selected tree nut oils was examined. The oils of almond, Brazil nut, hazelnut, pecan, pine nut, pistachio, and walnut were extracted using two solvent extraction systems, namely, hexane and chloroform/methanol. The chloroform/methanol system afforded a higher oil yield for each tree nut type examined (pine nut had the highest oil content, whereas almond had the lowest). The fatty acid compositions of tree nut oils were analyzed using gas chromatography, showing that oleic acid was the predominant fatty acid in all samples except pine nut and walnut oils, which contained high amounts of linoleic acid. The tocopherol compositions were analyzed using high-performance liquid chromatography, showing that alpha- and gamma-tocopherols were the predominant tocopherol homologues present; however delta- and beta-tocopherols were also detected in some samples. The oxidative stability of nonstripped and stripped tree nut oils was examined under two conditions, namely, accelerated autoxidation and photooxidation. Progression of oxidation was monitored using tests for conjugated dienes, peroxide value, p-anisidine value, and headspace volatiles. Primary products of

oxidation persisted in the earlier stages of oxidation, whereas secondary oxidation product levels increased dramatically during the later stages of oxidation. Hexanal was the major headspace aldehyde formed in all oxidized samples except walnut oil, which contained primarily propanal. Results showed that chloroform/methanol-extracted oils were more stable than hexane-extracted oils in both the accelerated autoxidation and photooxidation studies. Oils of pecan and pistachio were the most stable, whereas oils of pine nut and walnut were the least stable.

Psomiadou E. & Tsimidou M. (2002) "Stability of virgin olive oil. 1. Autoxidation studies." *J Agric Food Chem.* **50**(4), 716-21. Abstract. Virgin olive oil samples with similar oxidative stabilities and fatty acid compositions were stored for 24 months. Changes in the lipid substrate were followed by peroxide value and K(232) measurements. HPLC was used to evaluate changes in the alpha-tocopherol, pigment, and squalene contents. Total polar phenol content was measured colorimetrically. The loss of alpha-tocopherol and carotenoids was comparable with that of polar phenol content, suggesting an active participation in autoxidation. The limited role of squalene in autoxidation was further confirmed using an olive oil model and in the presence of alpha-tocopherol. Pheophytin a degradation was high, although spectrometric estimation of chlorophyll content did not indicate so. Evaluation of pheophytin a activity at three different levels of addition on the oil model indicated a concentration-dependent antioxidant role more pronounced at elevated temperatures, which could be partially due to the activity of certain degradation products.

Youdim,-K.A.; Deans,-S.G.; Finlayson,-H.J. (2002) "The antioxidant properties of thyme (*Thymus zygis* L.) essential oil: an inhibitor of lipid peroxidation and a free radical scavenger." *J Essen Oil Res* **14** (3), 210-215. Abstract. Antioxidants minimize the oxidation of lipid components in cell membranes by scavenging free radicals. However, imbalance between free radical production and removal tends to increase with age causing progressive damage. For the food industry it is of considerable interest to delay the autoxidation of food lipids, which cause the reduction in food quality, affecting color, taste, nutritive value, and functionality. A general orientation toward the use of natural compounds has stimulated research into the potential use of aromatic and medicinal plants as possible antioxidant replacements. This study characterized the antioxidant and pro-oxidant properties of thyme oil and a number of its components. The major components identified in thyme oil were found to inhibit ferric-ion-stimulated lipid peroxidation of rat brain homogenates, although none was as effective as the whole oil. The order of antioxidant activity was; thyme oil greater than thymol greater than carvacrol greater than gamma-terpinene greater than myrcene greater than linalool greater than p-cymene greater than limonene greater than 1,8-cineole greater than alpha-pinene. Both thyme oil and thymol were also found to inhibit tert-butyl-hydroperoxide-stimulated peroxidation and INT reduction by superoxide radicals generated by the xanthine-xanthine oxidase system. Of these compounds tested only p-cymene, 1,8-cineole and myrcene were found to exhibit pro-oxidant activity, albeit to a very small extent. Overall, the data suggest that

thyme oil possesses useful antioxidant properties that may be utilized in the food industry and as a dietary

Zainuddin A, Pokorný J. & Venskutonis R. (2003) "Antioxidant activity of sweetgrass (*Hierochloë odorata* Wahlnb.) extract in lard and rapeseed oil emulsions." *Nahrung* **46**(1), 15-7. Abstract. The antioxidant activities of sweetgrass (*Hierochloë odorata*) and sage (*Salvia officinalis*) extracts were studied in emulsions of lard and rapeseed oil using soy lecithin as an emulsifier, and addition of cupric acetate as an oxidation catalyst. The antioxidant activity was about the same in the two substrates. The stability against autoxidation was substantially increased by both sweetgrass and sage extracts and their combination. The stability was particularly high, if citric acid and/or ascorbyl palmitate were added to plant extracts.