N. Rettberg, S. Thörner and L.-A. Garbe

Bugging Hop Analysis – on the Isomerization and Oxidation of Terpene Alcohols during Steam Distillation

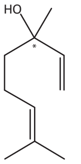
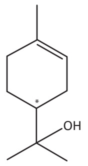
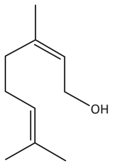
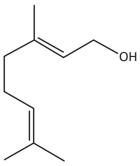
The major monoterpene alcohols of the hop essential oil undergo isomerization and oxidization reactions during steam distillation. Nerol and geraniol predominantly isomerize, their conversion gives rise to linalool and α-terpineol. Linalool is converted into isomeric furan and pyran linalool oxides, 2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyloct-7-en-2,6-diol. The chemical conversion of analytical targets during sample clean-up by steam distillation is objectionable and interferes with precise hop oil analysis.

Descriptors: hops, hop oil, mass spectrometry, analysis, linalool, oxidation

1 Introduction

Linalool, nerol, geraniol, and α-terpineol are the major monoterpene alcohols from hop essential oil. They have intense fruity and floral odors, and are the most valuable fragrances of many aroma hop varieties. The total concentration of terpene alcohols strongly depends on hop variety and ranges between 50 and 150 µg/g of dried hops. Linalool is commonly by far the most abundant compound [1]. In spite of their low concentration, terpene alcohols are of greatest relevance for brewers. They are more hydrophilic and less steam volatile than terpene hydrocarbons such as myrcene. Therefore, they are transferred from hops into beer, and cause pleasant hoppy and floral aroma notes. Linalool, nerol, geraniol and α-terpineol are structure isomers. Their molecular sum formulas accord, while their chemical structures and physiological properties i.e. their aroma impressions differ. Table 1 gives an overview on the chemical structures, systematic names (IUPAC) and the aroma properties of linalool, nerol, geraniol, and α-terpineol.

Table 1 Linalool, nerol, geraniol and α-terpineol are isomeric monoterpene alcohols. The asterisks in the chemical structures of linalool and α-terpineol indicate chiral centers. The odor qualities were determined using racemic mixtures [1]

	Linalool	**α-Terpineol**	**Nerol**	**Geraniol**
Chemical structure				
Formula	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{10}H_{18}O$
Systematic name (IUPAC)	3,7-Dimethyl-1,6-octadien-3-ol	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	3,7-Dimethyl-*cis*-2,6-octadien-1-ol	3,7-Dimethyl-*trans*-2,6-octadien-1-ol
Molecular weight	154,25	154,25	154,25	154,25
Aroma impression	floral, fresh, coriander	lilac	floral, fresh, green	floral, rose-like citrus

The analysis of terpene alcohols and other hop essential oils is a challenging discipline. The essential oils of hops are embedded in a complex matrix. They are usually separated by gas chromatography (GC) and quantified by internal standard methods. GC requires the removal of nonvolatile material. Despite of some sophisticated isolation techniques, such as solid-phase microextraction (SPME) [2, 3, 4], headspace traps [5], and thermal desorption [6], the hop oils are frequently extracted by steam distillation [7, 8, 9]. Volatile oils are separated from non-volatile material by boiling extracts, ground cones or pellets in water for several hours. The oils are evaporated, and either directly trapped in organic solvents (simultaneous distillation extraction [9]) or condensed as total oil [7, 8]. Steam distillation methods are generally criticized for indefinable analyte loss, artifact formation, and modifications of some compounds due to thermal impact [6]. It has been described that the essential oil composition alters due to steam distillation. Terpene alcohols and their esters undergo a variety of reactions [10]. However, this has not been studied in detail. To simplify the complexity, the terpene alcohol glycosides [11] and ester were not focus of this study. It is

Authors

Dipl.-Ing. Nils Rettberg, Technische Universität Berlin/Versuchs- und Lehranstalt für Brauerei in Berlin, (Fachgebiet Bioanalytik/Forschungsinstitut für Spezialanalytik), Seestr. 13, 13353 Berlin, Sarah Thörner (staatl. gepr. Lebensmittelchemikerin), Versuchs- und Lehranstalt für Brauerei in Berlin, Forschungsinstitut für Spezialanalytik, Seestr. 13, Berlin, Prof. Dr. Leif-Alexander Garbe, Technische Universität Berlin/Versuchs- und Lehranstalt für Brauerei in Berlin, (Fachgebiet Bioanalytik/Forschungsinstitut für Spezialanalytik), Seestr. 13, 13353 Berlin, leif-a.garbe@tu-berlin.de

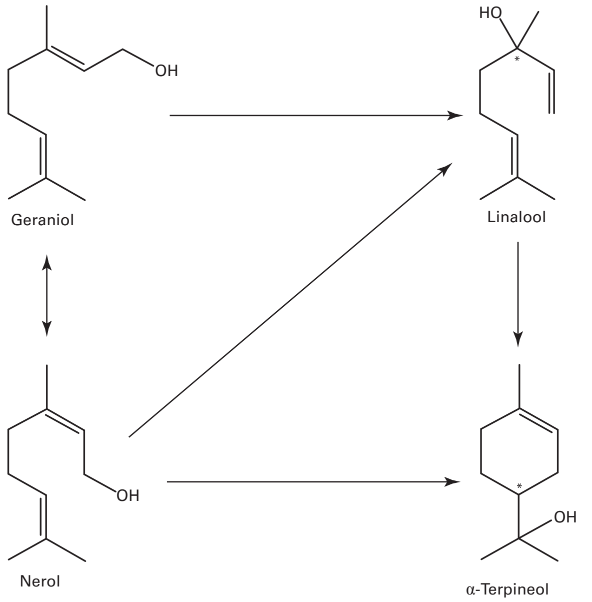


Fig. 1 **Nerol, geraniol, linalool and α-terpineol are structure isomers, that can be interconverted into each other (reactions indicated by arrows). The asterisks in the chemical structures of linalool and α-terpineol indicate chiral centers**

known that terpene hydrocarbons and alcohols are oxidized when exposed to oxygen [12]. Nerol and geraniol oxidation gives rise to aldehydes (neral and geranial), whereas linalool is converted into several oxidized derivates [13, 14]. In addition terpene alcohols are susceptible to acid catalyzed isomerization [15, 16, 17, 18, 19], as well as biotransformation by yeast and bacteria [1, 20, 21, 22, 23]. Figure 1 displays the structural similarity of the terpene alcohols. Arrows indicate the isomerization reactions that were observed in model wine (water-ethanol mixture, 10 abv, pH 2.9, 5 days at room temperature) [24]. Nerol and geraniol are cis-/trans-isomers, geraniol is the energetically favored compound. Both can isomerize and form linalool, nerol and linalool are able to form α-terpineol by ring closure.

Considering literature, terpene alcohols undergo a variety of transformations when exposed to oxygen, heat and acidic aqueous solutions. These conditions are present in steam distillation procedures, which are widely used in hop analysis. In the current paper we present compositional changes of the major hop terpene alcohols linalool, nerol, geraniol and α-terpineol during steam distillation. In a simplified model we monitored changes of individual terpene alcohols as well as their mixtures during boiling in water of pH 5.5. We identified and quantified products of isomerization and oxidation using gas-chromatography mass spectrometry (GC-MS).

2. Experimental

2.1 Chemicals

Analytical standards of linalool (97 %), α-terpineol (≥96 %), nerol (97 %), citral (≥95 %, geranial and neral mixture), geraniol (98 %) as well as cis- and trans-linalool oxide (mixture of 2-(5-methyl-5-vinyltetrahydro-1-furyl)-2-propanol isomers >97 %) were purchased from Sigma-Aldrich Chemie GmbH (D-89555 Steinheim). The absence of oxidation products was double checked by GC-MS. Further impurities of the individual terpene alcohols were considered within the calculations of resulting concentrations. Tert-butyl methyl ether (MTBE, ≥98,8 %), 0.1 NH_3PO_4 and anhydrous Na_2SO_4 were purchased from Carl Roth GmbH + Co. KG (D-76185 Karlsruhe). MTBE was further purified by distillation. Deionized water was drawn from a Merck Millipore Synergy® UV ultrapure water purification system (Merck Millipore, D-64293).

2.2 Steam distillation model

Individual terpene alcohols (each 20 µg of linalool, nerol geraniol and α-terpineol) as well as equimolar mixtures of linalool, nerol, and geraniol (20 µg in sum) were added to 500 mL two-neck round bottom flasks filled with 400 mL of ultrapure water and boiling chips. One neck of the flask was connected to a reflux condenser. The second was sealed by a rubber septum, that allowed sampling during distillation. pH was adjusted to 5.5 by addition of 0.1 N H_3PO_4. This pH was chosen because a pH of 5.2 and 5.8 is reached if 50-100 g ground hops is added to 2 L of deionized water. Reaction mixtures were heated to attain a rolling boil, then refluxed for up to four hours. Samples (20 mL) were withdrawn at start of boil and after 30, 60, 120, 180, 240 minutes and immediately cooled to room temperature. The aqueous solution was extracted twice with each 5 mL of MTBE. The organic layer was separated and dried over anhydrous Na_2SO_4. Excess solvent was gently removed to a volume of 1 mL by Vigreux-distillation. Remaining extract was transferred into sealed GC-vials and analyzed by GC-MS. Cold references of each compound and of the mixtures were prepared as described above, but extracted without heating/distillation. Distillation and extraction were carried out in triplicate determination. Distillation of water only was frequently carried out to exclude target carryover and systematic errors.

2.3 Analytical setup

GC-MS analysis was performed on Shimadzu GCMS-QP2010 Plus applying RXI® 5ms column (Restek, inner Ø = 0.25 mm, 0.25 µm film

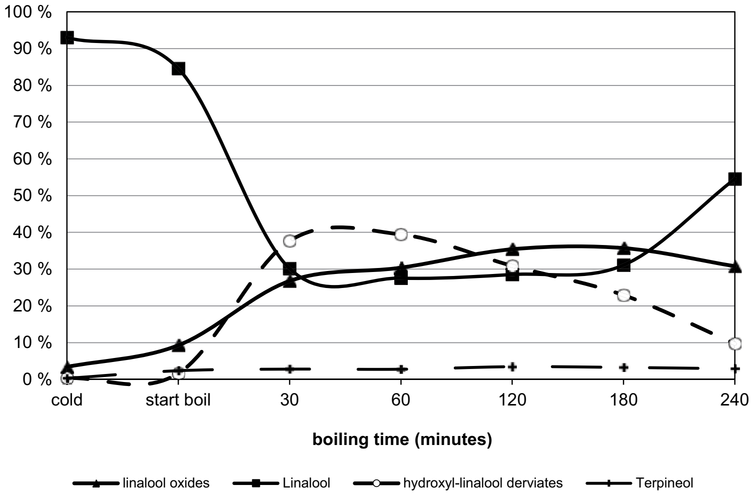


Fig. 2 **The concentration of linalool (area % of Scan-MS) decreases steam distillation. Linalool transformation yields in high concentration of linalool oxides and hydroxy-linalool derivates (2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyloct-7-en-2,6-diol)**

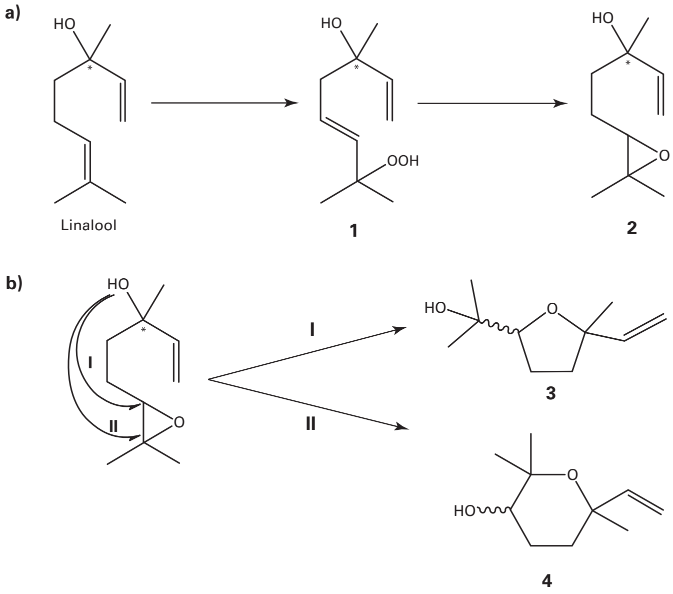


Fig. 3 **Proposed mechanism for the formation of furan and pyran oxides of linalool [13]. Reaction sequence a) shows the formation linalool epoxides (2), sequence b) the formation of isomeric furan (3) and pyran (4) linalool oxides. The asterisks indicate chiral centers, the sidled bond in 3 and 4 indicates the occurrence of two conformational isomers**

thickness, length = 30 m) for GC separation. This nonpolar phase showed very suitable performance to separate target compounds. Helium was used as carrier gas. Injector temperature was set to 240 °C, head pressure to 79.5 kPa. A sample volume of 0.5 µL was injected in split injection mode (split 10). Column oven temperature program was set at an initial temperature of 80°C (hold time 60 seconds), raised by a rate of 5 °C/minute until reaching 220 °C, then 50 °C/minute up to 300 °C. Ion source of quadrupole mass spectrometer (QP-MS) was adjusted to 200 °C, interface temperature to 250 °C. Analyte ionization was reached by electron impact ionization (EI). For data acquisition QP-MS was operated in scan mode (m/z=29-600).

2.4 Target identification and quantification

The identification of terpene alcohols and their reaction products was based on comparison of retention time and EI-mass spectra with reference standards. Furan linalool oxides, 2,6-dimethyloct-7-en-2,6-diol and 2,6-dimethyl-3,7-octadiene-2,6-diol were identified by means of their mass spectra in comparison with the NIST Database. Substance quantification was based on Scan-MS peak areas (total ion current). To simplify data comparison, concentrations of the individual compounds were given in percent of total volatiles. The GC-MS responses of all compounds were set to 1.

3 Results and discussion

3.1 Individual distillation trials

The monoterpene alcohols linalool, nerol and geraniol undergo various structural changes and are subject to oxidation during the steam distillation. Individual distillation experiments revealed that the concentrations of linalool, nerol and geraniol decreased rapidly when heated and isomers as well as oxygenated derivates were formed.

α-Terpineol was the only terpene alcohol that was stable during the four hour steam distillation procedure. An isomerization was not expected (Fig. 1), however also no oxidation products were traced.

Linalool was very reactive when exposed to heat. Figure 2 shows the major transformation products formed from linalool during steam distillation. The concentration of linalool was reduced to less to 55 % of the initial value (240 minutes heating). The lowest concentration, 28 % of total volatiles, was detected after 60 minutes.

Linalool conversion occurs jointly with increasing concentrations of linalool oxidation products. Furan linalool oxides (cis-/trans-2-(5-methyl-5-vinyltetrahydro-1-furyl)-2-propanol) were formed rapidly after the rolling boil was attained (start boil to 30 minutes). After 30 minutes of heating they accounted for 30 % of the detected volatiles. Their concentration remained constant at the following sampling points. The oxidation of linalool into linalool oxides has been described before [13], also they have been quantified in aged hop samples [25]. The proposed mechanism of linalool oxide formation is displayed in figure 3. It involves the formation of hydroperoxides (1, Fig. 3) and epoxides (2, Fig. 3). Both epoxide carbons

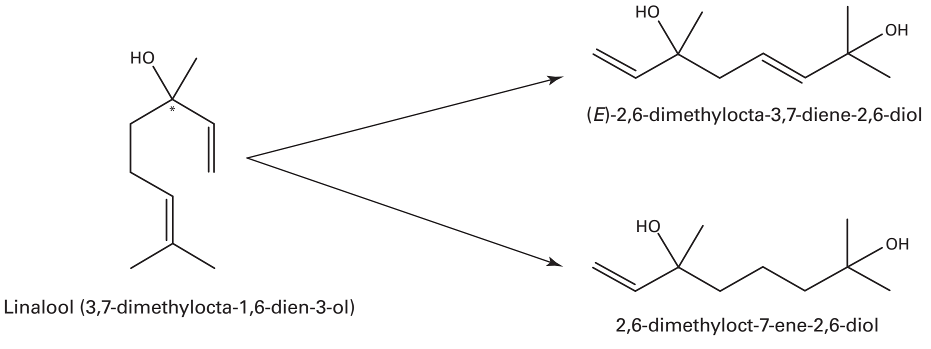


Fig. 4 **2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyloct-7-en-2,6-diol are hydroxyllinalool derivates**

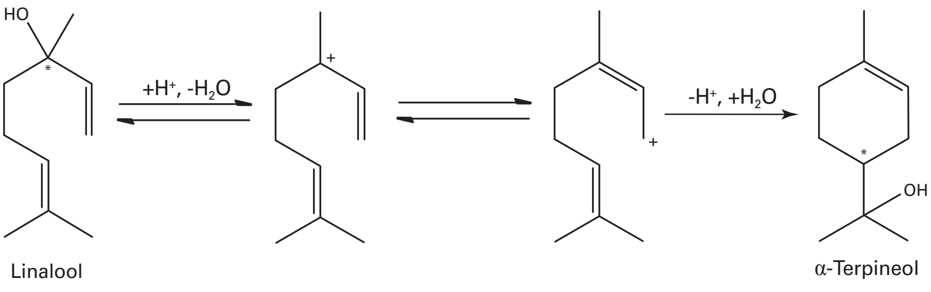


Fig. 5 **Proposed reaction scheme of the proton catalysed isomerization of linalool to α-terpineol. The asterisks indicate chiral centers**

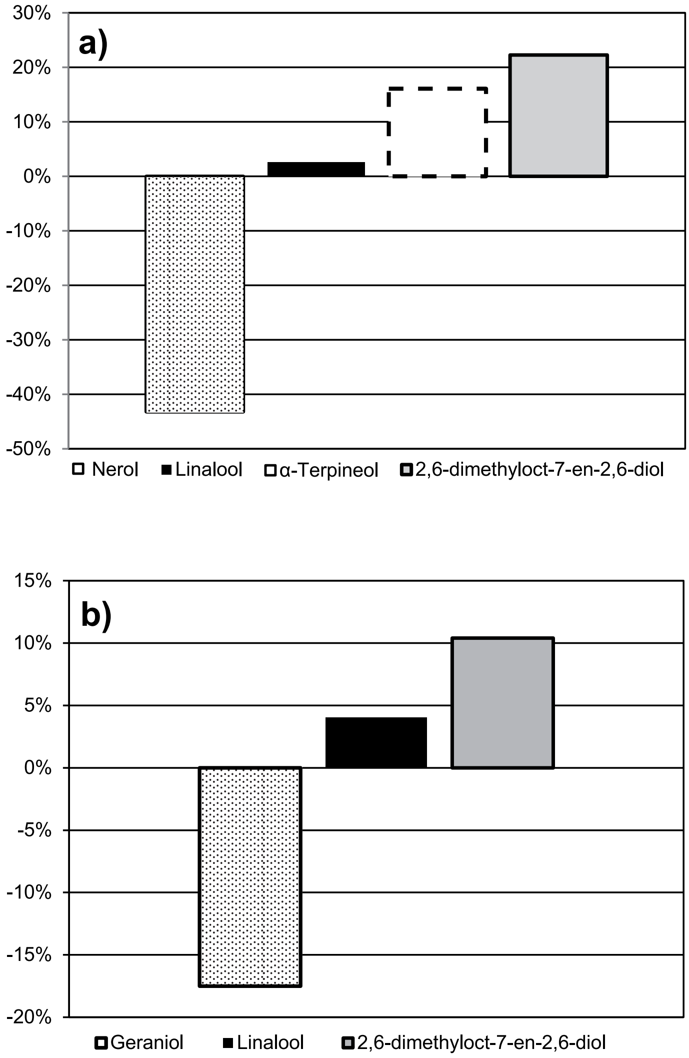


Fig. 6 **After 240 minutes of steam distillation nerol and geraniol concentrations strongly decreased and considerable amounts of transformation and oxidation products were detected**

are attacked by intramolecular hydroxyl groups, monomolecular nucleophilic substitution (SN_2) gives rise to the furan (3, Fig. 3) and pyran (4, Fig. 3) oxides of linalool, respectively. In contrast to the high concentrations of furan oxides of linalool, the pyran oxides were traced in maximum concentrations of 5 % drawn to the total volatiles. Both ring-structures are chemically stable, thus constant concentrations of linalool oxides during steam distillation were observed.

In addition to linalool oxides considerable amounts of hydroxyl-linalool derivates, 2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyloct-7-en-2,6-diol were detected. The chemical structures of both diols are displayed in figure 4, their formation from linalool has been observed before [26]. 2,6-Dimethyl-3,7-octadiene-2,6-diol is a degradation product of linalool hydroperoxides, 2,6-dimethyloct-7-en-2,6-diol is presumably formed by hydration reaction of linalool.

Their concentration (sum of both hydroxyl-linalool derivates) reached their maximum after 60 minutes of boil, decreasing concentrations were observed with persisting boil. The amounts of hydroxyl-linalool derivates seem to be connected with the increase of linalool observed with persisting boil. Presumably the hydroxyl-linalool formation is reversible and diols decompose to form linalool again. The isomerization of linalool into α-terpineol could be observed at very low rates. α-terpineol was predominately formed during the heating phase, and remained roughly constant throughout refluxed boiling (≤ 3 %). A proposed reaction scheme of a proton-catalyzed isomerization is given in figure 5.

3. Geraniol and nerol are cis-/trans-isomers. They were less reactive than linalool under steam distillation conditions. Their distortion is displayed in figure 6. The oxidation of both alcohols resulted in slightly increasing concentrations of their corresponding aldehydes neral and geranial (2-4 %, data not included in Fig.6). Nerol was predominantly converted into 2,6-dimethyloct-7-en-2,6-diol (20 %), α-terpineol (16 %) and linalool (5 %) (a, Fig. 6). Also traces of geraniol (2 %) were detected. Geraniol is the energetically favored trans-isomer of nerol (Fig. 1 and Table 1). During steam distillation 20 % of geraniol were converted into 2,6-dimethyloct-7-en-2,6-diol (12 %) and linalool (5 %) (b, Fig. 6). In addition to both major products geranial (1-2 %) as well as traces of 2,6-dimethyl-3,7-octadiene-2,6-diol, β-citronellol, citronellal could be detected. The observed chemical interconvertions of terpene alcohols during steam distillation (pH 5.5) are similar to the pathways found in model wine of pH 2.9 [23]. In addition to isomerization oxidative reactions gain importance due to heat impact. The occurrence of 2,6-dimethyloct-7-en-2,6-diol and dimethyl-3,7-octadiene-2,6-diol is obviously connected to the secondary oxidation of linalool (Fig. 2 and Fig. 4).

3.2 Distillation of linalool, geraniol and nerol mixtures

The results of the individual terpene alcohol boiling trails were confirmed by steam distillation of equimolar mixtures of linalool, nerol and geraniol. Figure 8 shows their distribution and abundant transformation products over a boiling time of 240 minutes. The bar chart diagram in figure 7 suitably displays that the concentrations of nerol, geraniol and linalool decrease, while the concentrations of oxidized linalool derivates and α-terpineol increase. Geraniol degradation is obviously slower and less distinct compared to linalool and nerol. These findings match with the results gained from individual boiling experiments (Fig. 4). During steam distillation of the mixture considerable amounts of linalool oxides develop within the first 30 minutes of boil. After four hours they sum up to approx. 15 % of the total volatiles. The hydroxy-linalool derivates 2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyloct-7-en-2,6-diol increased as well. They accumulated to approx. 20 %, and exceeded the concentrations of linalool and nerol. Besides those mayor compounds (Fig. 5 and Fig. 6) geranial, neral, and traces of β-citronellol, as well as 3,7-dimethyloct-6-en-3-ol (dihydro-linalool) could be detected. In sum their concentration was below 3 % of the total peak area of GC-Scan-MS.

3.3 Assessment of the steam distillation model

The results displayed in 3.1 and 3.2 definitely indicate the transformation of terpene alcohols during reflux boiling in a model solution (aqueous, pH 5.5). Because some terpene hydrocarbons and alcohols can be converted into each other – enzymatic and

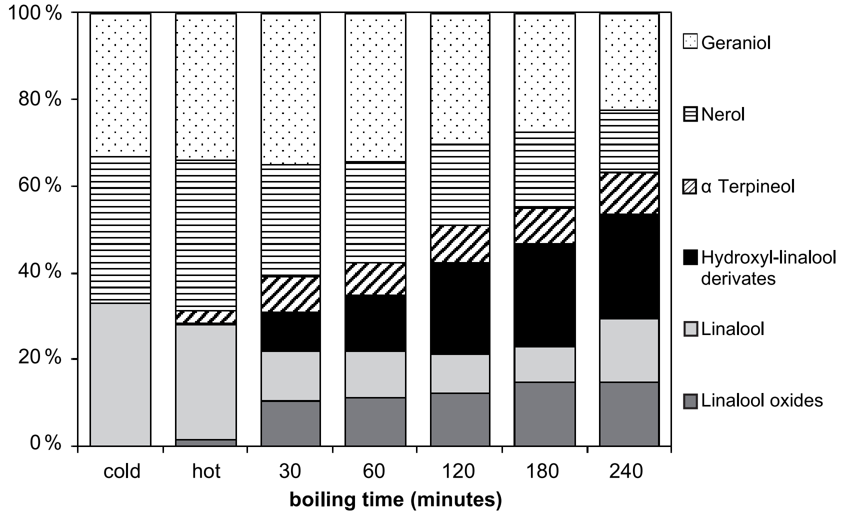


Fig. 7 **Time dependent composition of volatiles during steam distillation of equimolar mixtures of linalool, geraniol and nerol in water (pH 5.5). Bar charts indicate the conversion of linalool, geraniol and nerol: Their concentration decreases while the amounts of oxidized products increase with boiling time**

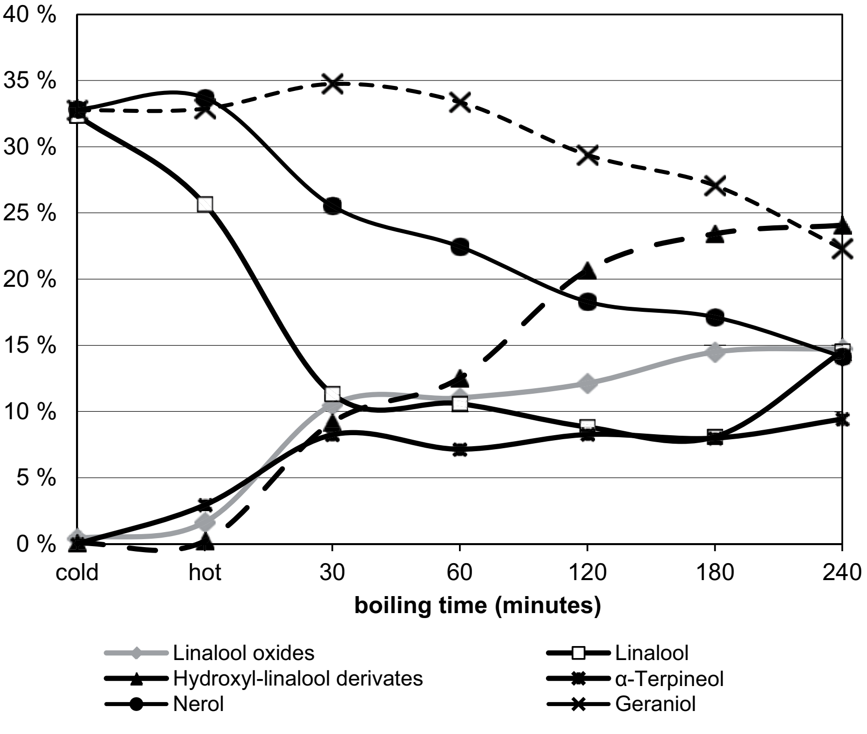


Fig. 8 **Time dependent composition of volatiles during steam distillation of equimolar mixtures of linalool, geraniol and nerol in water (pH 5.5). Their concentrations decrease while oxidized derivates and α-terpineol increase**

chemically – there is no chance to monitor reactions of individual compounds in hop samples without the use of isotope labeling. Since isotope standards of hop constituents are not commercially available, we established the presented distillation model. The model differs in two important points from distillation in hop analysis: Firstly, it excludes matrix effects: Especially antioxidants and lipids present in hops tentatively stabilize hop oils and minimize oxidative impact. The release of monoterpene alcohols bound as glycosides [11] or esters will interfere hop analysis as well and will be focused in future investigations.

Secondly, reflux boiling returns evaporated oils back into the hot liquid instead of removing them. However, it enabled a detailed examination of the routes of chemical conversion of important analytical targets (linalool, geraniol and nerol). We have chosen the reflux boiling method because the terpene alcohols are not very steam volatile and are exposed to high thermal impact during distillation. In contrast to many terpene hydrocarbons intensive boil and distillation for hours are needed to evaporate them quantitatively. The used experimental setup enabled a convenient sampling during distillation. We consider our model to be highly acceptable in order to prove and monitor the instability of important analytical targets in hop analysis. A detailed survey on the reactions including hop matrix, as well as the synthesis of stable isotope labeled terpene standards is currently under investigation.

4 Summary and conclusion

During steam distillation the major monoterpene alcohols of the hop essential oil undergo several chemical reactions. Nerol and geraniol predominantly isomerize, linalool is very susceptible to oxidation. A distortion of the essential oil composition during isolation by steam distillation can significantly interfere hop analysis and cause imprecise quantification of key compounds. The oxidation of linalool is of special concern. Linalool is usually the most abundant terpene alcohol and regarded as major quality criteria of hops. Linalool oxide and hydroxyl-linalool formation during analysis can lead to the detection of apparently lowered linalool concentrations. To improve hop analysis we recommend the use of isolation techniques with minimized thermal impact, as well as the use of stable isotope internal standards. Stable isotope standards such as D5-Linalool indicate and cover chemical conversions of target compounds during analysis [27]. They permit to distinguish e.g. whether linalool oxides originate from hops or are formed during analysis. Thus they can boost the precision of hop analysis even the conventional steam distillation method is applied.

5 References

1. Takoi, K.; Koie, K.; Itoga, Y.; Katayama, Y.; Shimase, M.; Nakayama, Y. and Watari, J.: Biotransformation of Hop-Derived Monoterpene Alcohols by Lager Yeast and Their Contribution to the Flavor of Hopped Beer, Journal of Agricultural and Food Chemistry **58** (2010), pp. 5050-5058.
2. Field, J. A.; Nickerson, G.; James, D. D. and Heider, C.: Determination of Essential Oils in Hops by Headspace Solid-Phase Microextraction, Journal of Agricultural and Food Chemistry **44** (1996), pp. 1768-1772.
3. Kovačevič, M. and Kač, M: Solid-phase microextraction of hop volatiles – Potential use for determination and verification of hop varieties, Journal of Chromatography A **918** (2001), pp. 159-167.

4. Steinhaus, M.; Fritsch, H. T. and Schieberle, P.: Quantitation of (R)- and (S)-Linalool in Beer Using Solid Phase Microextraction (SPME) in Combination with a Stable Isotope Dilution Assay (SIDA), Journal of Agricultural and Food Chemistry **51** (2003), pp. 7100-7105.
5. Aberl, A. and Coelhan, M.: Determination of Volatile Compounds in Different Hop Varieties by Headspace-Trap GC/MS-In Comparison with Conventional Hop Essential Oil Analysis, Journal of Agricultural and Food Chemistry **61** (2012), pp. 2785-2792.
6. Eri, S.; Khoo, B. K.; Lech, J. and Hartmann, T. G.: Direct Thermal Desorption-Gas Chromatography and Gas Chromatography-Mass Spectrometry Profiling of Hop (*Humulus lupulus* L.) Essential Oils in Support of Varietal Characterization, Journal of Agricultural and Food Chemistry **48** (2000), pp. 1140-1149.
7. European Brewery Convention: Analytica-EBC; Fachverlag Hans Carl: Nuremberg, Germany, 2006; Section 7 Hops, Methods 7.10 and 7.12
8. American Society of Brewing Chemists: Methods of Analysis, 8th ed.; ASBC: St. Paul, MN, 1992; Hops-**13**, 1-2.
9. Nickerson, G. B. and Likens, S.T.: Gas Chromatography evidence for the occurrence of hop oil components in beer, Journal of Chromatography A **21** (1966), pp. 1-5.
10. Picket, J. A.; Coates, J. and Sharpe, F. R.: Distortion of essential oil composition during isolation by steam distillation. Chemistry and Industry (1975), pp. 571-572.
11. Kollmannsberger, H., Biendl, M., and Nitz, S.: Occurrence of glycosidically bound flavor compounds in hops, hop products and beer. Monatsschrift fuer Brauwissenschaft **59** (2006), pp. 83-89.
12. Dieckmann, R. H. and Palamand, S. R.: Autoxidation of some constituents of hops. I. Monoterpene hydrocarbon, myrcene, Journal of Agricultural and Food Chemistry **22** (1974), 498-503.
13. Hagvall, L.; Bäcktorp, C.; Svensson, S.; Nyman, G.; Börje, A. and Karlberg, A.-T.: Fragrance Compound Geraniol Forms Contact Allergens on Air Exposure. Identification and Quantification of Oxidation Products and Effect on Skin Sensitization, Chemical Research in Toxicology **20** (2007), pp. 807-814.
14. Sköld, M.; Börje, A.; Harambasic, E. and Karlberg, A.-T.: Contact Allergens Formed on Air Exposure of Linalool. Identification and Quantification of Primary and Secondary Oxidation Products and the Effect on Skin Sensitization, Chemical Research in Toxicology **17** (2004), pp. 1697-1705.
15. Sefton, M.A.; Francis, I.L. and Williams, P.J.: The free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Semillon, Australian Journal of Grape and Wine Research (1996), pp. 179-183.
16. Rapp, A.; Güntert, M. and Ullemeyer, H.: Über Veränderungen der Aromastoffe während der Flaschenlagerung von Weißweinen der Rebsorte Riesling. Food Research and Technology **180** (1985), pp. 109-116.
17. Ramirez Ramirez, G.; Lubbers, S.; Charpentier, C.; Feuillat, M.; Voilley, A. and Chassagne, D.: Aroma Compound Sorption by Oak Wood in a Model Wine, Journal of Agricultural and Food Chemistry **49** (2001), pp. 3893-3897.
18. Ohta, T.; Morimitsu, Y.; Sameshima, Y.; Samuta, T. and Ohba, T.: Transformation from geraniol, nerol and their glucosides into linalool and α-terpineol during shochu distillation. Journal of Fermentation and Bioengineering **72** (1991), pp. 347-351.
19. Demyttenaere, J.C.R.; del Carmen Herrera, M. and De Kimpe, N.: Biotransformation of geraniol, nerol and citral by sporulated surface cultures of *Aspergillus niger* and *Penicillium sp.*, Phytochemistry **55** (2000), pp. 363-373.
20. Seaton, J. C.; Moir, M. and Sugget, A.: The refinement of hop flavour by yeast action, The Institute of Brewing, Australia and New Zealand, Section: Sydney, Australia (1982), pp. 117-124.
21. Lam, K. C.; Foster, R. T. and Deinzer, M. L.: Aging of hops and their contribution to beer flavor, Journal of Agricultural and Food Chemistry **34** (1986), pp. 763-770.
22. King, A. and Dickinson, J. R.: Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis.*, Yeast **16** (2000), pp. 499-506.
23. King, A. and Dickinson, J. R.: Biotransformation of hop aroma terpenoids by ale and lager yeasts. FEMS Yeast Research **3** (2003), pp. 53-62.
24. Pedersen, D. S.; Capone, D. L.; Skouroumounis, G. K.; Pollnitz, A. P. and Sefton, M. A.: Quantitative analysis of geraniol, nerol, linalool, and α-terpineol in wine, Analytical and Bioanalytical Chemistry **375** (2003), pp. 517-522.
25. Tressl, R.; Friese, L.; Fendesack, F. and Koeppler, H.: Studies of the volatile composition of hops during storage, Journal of Agricultural and Food Chemistry **26** (1978), pp. 1426-1430.
26. Bock, G.; Benda, I. and Schreier, P.: Biotransformation of Linalool by Botrytis cinerea, Journal of Food Science **51** (1986), pp. 659-662.
27. Garbe, L.-A. and Rettberg, N. The Power of Stable Isotope Dilution Assays in Brewing, BrewingScience – Monatsschrift fuer Brauwissenschaft, 64 (2011), pp. 140-150.

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