Report on determination of volatile compounds in different hop varieties by heads...

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The research article entitled “ Determination of volatile compounds in different hop varieties by headspace-trap GC/MS -In comparison with conventional hop essential oil analysis” is authored by Anita Aberl and Mehmet Coelhan of the Research Center for Brewing and Food Quality, Freising-Weihenstephan, Germany. This article appeared in the “ Journal of Agricultural and Food Chemistry” of ACS publications in the year 2012 and attempted to compare the efficiency and accuracy of Head Space (HS)-trap method coupled with GC/MS and conventional essential oil analysis method. Humulus lupulus L., commonly known as hop is responsible for the bitter flavor and ‘ hoppy’ aroma of the beer. The chemical constituents of hop include bitter substances and essential oils. More than 400 chemicals have been reported from the hop essential oils and are classified into two structural categories viz. Hydrocarbons and oxygenated compounds. Hydrocarbons can be further divided into monoterpenes and sesquiterpenes.

Myrcene and linalool are the primary odorous chemicals of hop essential oil. However, only linalool is responsible for the aromatic flavor of beers because myrcene evaporates during wort boiling. The components of the essential oil of hops are analyzed and quantified by several methods. Steam distillation, extraction with organic solvents, extraction with carbon dioxide and thermal desorption methods are generally employed for the isolation of essential oil and analysis of its chemical constituents. However, all of these methods have certain limitations such as extensive sample preparation, requirement of specialized equipment, long extraction time, analyte loss during processing and damage of gas chromatography (GC) columns by the non-

volatile residues of hops.

In the Head Space (HS)-trap technology, the sample is heated inside a sealed vial until equilibrium. This is followed by pressurization of the vial contents by a carrier gas. Analyte vapors are extracted from the sample vial by cooled adsorbent trap and a dry carrier gas is passed through the trap to remove the moisture from the analyte. The analyte is then thermally desorbed and transported to the GC column by the carrier gas. This method is simple, sensitive & rapid and involves minimal analyte losses. The detection limits of the analyte can be improved by repeating trap enrichment cycle. In the present study, HS-trap method was combined with GC-MS for the determination of volatile constituents of hop.

For this research, 24 commercial samples of different varieties of hops were procured and stored at -24 ºC until analysis. Standards of monoterpenes and sesquiterpenes were purchased from different companies and were used in the analysis. Standard calibration curves were generated for the constituents of hops. The sample preparation of hops for HS-trap analysis involved extraction of 2. 0 sample (pellets or cones) of hops in 18. 0 g ethanol by ultra-sonication at 55 ºC for 45 min. A 20. 0 mg sample of supernatant was mixed with 5. 0 ml of pure water (after cooling of the extract). This solution was transferred to an HS vial and spiked with 6 µL of internal standard.

Another sample of hops was prepared for analysis of volatile constituents by EBC (conventional essential oil analysis) method. For this, a 25. 0 g sample of hops was placed in a round-bottom flask (2. 0 L) and 1. 25 L of pure water was added to it. Distillation was carried out to extract the hops oil. This oil was used for making a concentration of 700 ng oil per µL of ethanol. Internal

standards n-nonane and linalool-D3 were added the above solution before GC/MS. GC/MS analysis of hops oil was performed using Thermo trace ultra GC system coupled to DSQ II Mass spectrometer. A turbo Matrix HS-40 trap was used as sampler and helium was used as the carrier gas. All samples were analyzed in duplicate.

More than 65 volatile constituents of hops were identified by MS library search after HS-trap and conventional method of analysis. Of these 65, 21 could be quantified using analyte specific calibration curves. Figure 1 presents the representative chromatogram of Hallertauer Magnum variety of hops. Peaks of the quantified compounds are labeled on the chromatogram.

Figure 1. HS-trap GC/MS chromatogram of the hop variety Hallertauer Magnum. Peaks: 1, n-nonane (internal standard); 2, isobutylisobutyrate; 3, methylhexanoate; 4, α-pinene; 5, β-pinene; 6, myrcene; 7, methylheptanoate; 8, limonene; 9, 2-nonanone; 10, linalool + linalool-D3; 11,

methyloctanoate; 12, 2-decanone; 13, methylnonanoate; 14, 2-undecanone; 15, methyldecanoate, 16, damascenone; 17, 2-dodecanone; 18, β-caryophyllene; 19, α-humulene; 20, ethyldodecanoate; and 21, caryophyllene oxide.

Reproducibility of the HS-trap method was proved by calculating the coefficient of variation for all the analytes. The coefficient of variation for the analyzed compounds was between 1. 3 to 5. 1% and the recoveries of these compounds were between 83. 4 to 119. 4% by the HS trap method (when the recovery was assumed to be 100% by EBC method). Both the methods viz. HS-trap and hop essential oil analysis gave comparable results for almost

all analytes. Compounds with boiling points lower than 215 ºC showed better correlations between the two methods.

It is notable that HS-trap method showed higher concentrations of compounds with relatively low boiling points (such as monoterpenes). On the contrary, hop essential oil analysis method showed higher concentrations of compounds with boiling points higher than 228 ºC (including all sesquiterpenes). For example, the concentration of α-humulene as obtained by oil analysis method was 30% higher than with HS-trap method. However, caryophyllene oxide was an exception to this rule. The concentration of caryophyllene oxide as revealed by HS-trap method was 2. 9-fold higher than with the other method. This was because of the substantial decomposition of caryophyllene oxide during steam distillation. Deviations in the concentrations of high boiling components may be reduced by the use of isotopically-labeled internal standards.

This study revealed that the essential oil content in the hops was dependant on the species and bitter hops contained higher levels of essential oils. However, it is not advisable to distinguish the hop varieties on the basis of total essential oils because the amount and compositions of these oils vary with climatic and soil conditions. The analyses of the hop essential oil components revealed that compounds with same chemical (structural) group correlated with each other. This may be because of common biosynthetic stages or inter-conversion of these compounds.

Overall, HS-trap method gave accurate results for highly volatile compounds and epoxides. The accuracy of the method may further be increased by extending the number of analyzed compounds. This method is highly

sensitive, rapid & easy to perform and the results of quantification of hop essential oil components by this method were comparable to the conventional method. Further, this method may be applied for the analysis of volatile oils from other plant species.

Reference

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