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Hop Volatile Compounds (Part I): Analysis of Hop Pellets and Seasonal Variations

The measurement of the hop volatiles targets the flavour-active components, mainly terpene and sesquiterpene alcohols, that are able to create the typical hoppy flavour in beer. These substances are both indicator substances as well as key components of the flavour. This paper presents the method of analysis via aqueous extraction, water steam distillation and detection by GC-FID. Furthermore data is provided, describing the relationship between alpha acids content and oil content compared to actual flavour component levels. Neither alpha acids content nor oil content show a reliable correlation to the actual content of flavour compounds. It is therefore proposed to dose hop pellets according to the actual level of hop flavours and not according to the currently used dosage based on alpha-acids content. Part 2 of this paper presents data on transfer rates.

Descriptors: hop, hopping, beer flavour, hop aroma, analysis, gaschromatography, analytical method, volatile compounds

1 Introduction

Until today several hundred aroma compounds are identified from hop oil. They can be divided into several classes. The biggest group are the hydrocarbons which are subdivided into mono- and sesquiterpenes as well as aliphatic hydrocarbons. Approx. 30 % of the oil composition are oxygen containing substances. [1] Because of the different processing and dilution steps during brewing the hop aroma of beer is very different from the aroma of the hop product [2, 3]. Myrcene, the major compound of the hop oil can not be found in the final beer [1] unless cold hopping is used. So the fine hoppy flavour of a beer flavoured with hops in the kettle/whirlpool is due to other compounds.

Because of the highly significant correlation between the concentration and the perceived hop flavour the terpen alcohol linalool was claimed as lead substance for the hoppy flavour by Kaltner and Fritsch [4, 5, 6]. A hoppy flavour in beer is being created by a late addition in the boil or in the whirlpool. This results in a pleasant hop aroma because of minimized evaporation losses of the hop aroma compounds [7, 8, 5, 6, 9]. Depending on the hop variety used the character of the hop flavour of the final beer can vary in different ways (citrussy, herbal, spicy, flowery, fruity) [6, 10, 11]. Typically, the last hopping is dosed according to the alpha acid content of the hops. It would be more logical to dose it according to the aroma content, because a bitter acid based dosage can not guarantee a constant hop aroma in beer. For realizing a constant hop flavour a simple analysis for the most common hop aroma compounds is needed. This analysis will be presented in this paper. Based on this analyis, an overview on yearly variation between alpha acid content and hop oil content will be presented.

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Tables and Figures see Appendix.

2 Analysis

The analysis is based on the various water vapour distillation methods published by Mebak [12].

Principle:

The sample's volatile compounds are expelled by water vapour distillation. The ethanolic distillate is alkalized and furthermore being saturated with NaCl. The volatile compounds are then extracted via Dichloromethane; the volume of the organic phase is further on reduced by a nitrogen flow.

The addition of ammoniac is used to separate organic acids as they are often accountable for coelutions with relevant substances.

2.1 Instruments and Materials

Instruments:

- Water bath (shaking) Julabo SW-20C
- Centrifuge Sigma 6K-15 with cooling
- pH-meter Inolab
- Hewlett Packard HP 5890 with Split- /Split less-injector, 2 capillary columns (HP Innowax (Polyethylene Glycol) 60 m

* 0.20 mm * 0.40 µm; HP Ultra 2 (5 % Ph.- 95 % Me-Si) 60 m*0.20 mm * 0.33 µm) and 2 flame ionization detectors.

- Hewlett Packard HP 7673 A Automatic Sampler
- Heraeus-centrifuge with cooling: Varifuge RF
- Turbula-shaker
- Distillation unit: Büchi K-314

Chemicals

All chemicals used were of GC- or p.a. quality. Suppliers: Sigma-Aldrich, Roth, Riedel-de Haën, Merck.

Auxiliary materials

- Glycerinmonostearate (ICN-Biochemicals No. 195334) as Anti-foam
- Dichloromethane p.A. (Riedel-de Haën, No. 3222), redis tilled
- NaCl p.A. (Merck 6404.5000)
- Ammonia 25 % w/w p.A. (Merck 5432.5000)
- Nitrogen 5.0 (Linde)
- NaOH 1 mol/L or Phoshoric Acid 1 mol/l for pH-correction
- Ethanol p.A. (Fluka)
- Potassium bisulfite p.A. ($K_2S_2O_5$) (Merck 5057.5000)

Internal Standard

The internal standard solution consists of phenylmethanol (Sigma-Aldrich, 1500 mg/l) and heptanoic acid methyl ester (Sigma-Aldrich, 100 mg/l) in Ethanol p.a..

2.2 Sample Preparation

Cold extraction of the sample

1 g of hop pellets is weighed into a beaker and 150 ml H_2O dist. at 20°C is added. The pH is adjusted to 5.4 using a pH-meter and phosphoric acid The suspension is then being shaked for 60 min in the Julabo SW-20C water bath at 60 min^{-1} at 80 °C in a water bath and cooled down quickly afterwards. Next it is being transferred into a tumbler and centrifuged for 15 min. at 20°C with 3000 U/min^{-1}. The supernatant is decanted into a 100 ml volumetric flask.

Water vapour distillation

9 mL Ethanol p.A. and 1 mL ISTD are added to the volumetric flask. A spatula's tip of antifoam is provided in a distillation tumbler; the content of the volumetric flask is completely poured into this distillation tumbler and distilled afterwards. The distillation takes 5 minutes at the conditions preset in the Büchi K-314 Unit. A little more than 100 ml of the distillate is collected in an ice-cooled volumetric flask and then adjusted to exactly 100 ml. After thorough homogenization 20 ml of the distillate are removed (20 mL volumetric pipette).

Extraction

22 g NaCl are weighed into a screw top tumbler. The remaining 80 ml of distillate, 1 g potassium bisulfite and 0,5 ml Dichloromethane are added; the tumbler is tested for tightness. The tumbler is being shaked for 30 mins in the Turbula-shaker and subsequently centrifuged for 15 mins at 0 °C and 2400 /min^{-1}.

After siphoning off some parts of the aqueous phase with a water jet pump, the organic phase (in the form of a Dichloromethane-bead) is transferred into a 1 ml vial with the aid of a Pasteur-pipette. The organic phase is then reduced to ~150 µl by a nitrogen flow and transferred into a conus vial.

2.3 Gaschromatographtic Conditions

Table 1 shows the gaschromatographic conditions.

2.4 Calibration

The calibration is done by addition of the reference substances in six different concentrations and reporting of the relative peak areas. The substances are weighed into a 5 %vol. Ethanol/Water solution. During the dilution series the ethanol content is kept at the same level. To create a similar matrix, hop pellets are prepared as noted above and cooked out thoroughly for 30 mins to reduce volatile compounds before the standards are added. 1ml of each corresponding dilution sample are added, resulting in an Ethanol content of 0,00033 % Vol. in the calibration solutions. The added concentrations are plotted over the corresponding relative peak areas. Evaluation is done by linear regression analysis. The slope of the regression graph then denotes the calibration factor of each respective substance. Figure 1 shows an exemplary calibration plot for Geraniol.

2.5 Repeatability

Table 2 shows the repeatability of the analysis as coefficients of variation (CoV) with n = 10.

3 Comparison of hop volatile compounds in dependency of alpha-acid and oil content

3.1 Alpha-acid content and hop volatile content

Table 3 shows linalool content versus α-acids content, measured according to EBC 7.5 [13] for the hop varieties "Hallertauer Smaragd", pellets type 90 (abbr. P90 HSD) and "Tettnang Tettnanger", pellets type 45 (abbr. P45 TTE) for the crops of year 2005 (abbr. C05) and 2006 (abbr. C06).

Linalool varies greatly in both hop varieties and also by years. Dosing the flavour hops according to α-alpha acid content (EBC 7.5) would result in significantly different levels of actual added hop flavour volatile compounds. At the same dosage of 5 mg α-alpha acids, the brewer would add 104.0 µg Linalool/5 mg α-alpha acids for P45 TTE C05, 67.5 µg Linalool/5 5 mg α-alpha acids for P45 TTE C06(1) and 64.0 µg Linalool/5 mg α-alpha acids for P45 TTE C06(2). Thus the dosage of volatile compound would be just 61 % of P45 TTE C05 compared with P45 TTE C06(2). A different hop flavour in beer is surely to be expected then.

3.2 Oil content and hop volatile content

Table 4 shows linalool content of the hop variety "Tettnang Tettnanger" against oil content (EBC 7.10) and α-acid content (EBC 7.5).

Data from this table demonstrates, that total oil content gives no guarantee to reach standardized hop flavour either. The oil content is varying strongly over the years and the linalool content neither does remain the same. The linalool content thus shows differences of up to 33 % depending on the crop year. The values for α-acids supports the findings of 3.1.

4 Perspective

The direct measurement of hop volatile compounds after an aqueous extraction at temperatures and pH-values, which are comparable to the brewing process, results in sensible, praxis-relevant data. These data, as it will be shown in part 2 of this paper [14], are a suitable tool for brewers to get a standardized hop flavour in beer as this analysis takes the possible influences from crop year and other agronomical conditions into account. Neither alpha-acids content, nor oil content can provide this, as the actual flavour volatile transfer to the beer will vary greatly if the dosage is based on those. It is recommended to base the aroma dosage on the data derived from this analysis.

5 Literature

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Appendix

Table 1 Gaschromatographic conditions

		Temperatures	Flow rates
Injector	Pressure: 150 kPa	250 °C	
Injection volume	4 µl		
Carrier gas	Hydrogenium 5.0		1,9 ml/min.
Septum-Purge			5,8 ml/min.
Split	01:07		61,9 ml/min
Capillary column I	HP Innowax (Polyethylene Glycol) 60 m * 0,20 mm * 0,40 µm	4 min.: 60 °C 5 °C/min. to 220 °C 40 min.: 220 °C	
Capillary column II	HP Ultra 2 (5 % Ph.- 95 % Me-Si) 60 m * 0,20 mm * 0,33 µm	4 min.: 60 °C 5 °C/min. to 220 °C 40 min.: 220 °C	
Detector	2 x FID	250 °C	
Detector gases	Hydrogenium 5.0 Synthetic Air Nitrogenium 5.0 (Make-up-Gas)	 16,5 ml/min.	35,4 ml/min. 400 ml/min.
Reporting	Area modus with ISTD		

Table 2 Coefficients of variation

Coefficient of Variation	[%]
Geraniol	11,7
Terpineol	8,2
Nerol	5,7
Humulen	22,7
Linalool	11,9

Table 3 Linalool and α-acids content in varieties “Hallertauer Smaragd” and “Tettnang Tettnanger”

	P90 HSD C05(1)	P90 HSD C05(2)	P90 HSD C06
Linalool [µg/g]	49.5	66.0	58.5
EBC 7,5 [%]	7.0	6.6	6.8
Lin./EBC	7.1	10.0	8.6
	P45 TTE C05	**P45 TTE C06(1)**	**P45 TTE C06(2)**
Linalool [µg/g]	54.0	40.5	34.5
EBC 7,5 [%]	2.6	3.0	2.7
Lin./EBC	20.8	13.5	12.8

Table 4 Linalool content, Oil content and α-acids content in the variety "Tettnang Tettnanger (Pellets Type 90)

	TTE 2004	TTE 2005	TTE 2006	TTE 2007
EBC 7.5	5,1	5,1	2,4	4,0
Total Oil (ml/100g)	0,4	0,6	0,4	0,7
% Linalool	0,6	0,6	0,8	0,8
Linalool [µg/g]	19,2	28,8	25,6	44,8
Linalool/Total Oil	48,0	48,0	64,0	64,0
Linalool/EBC 7.5	3,8	5,7	10,7	11,2
Total Oil/EBC 7.5	0,0208	0,0208	0,0156	0,0156

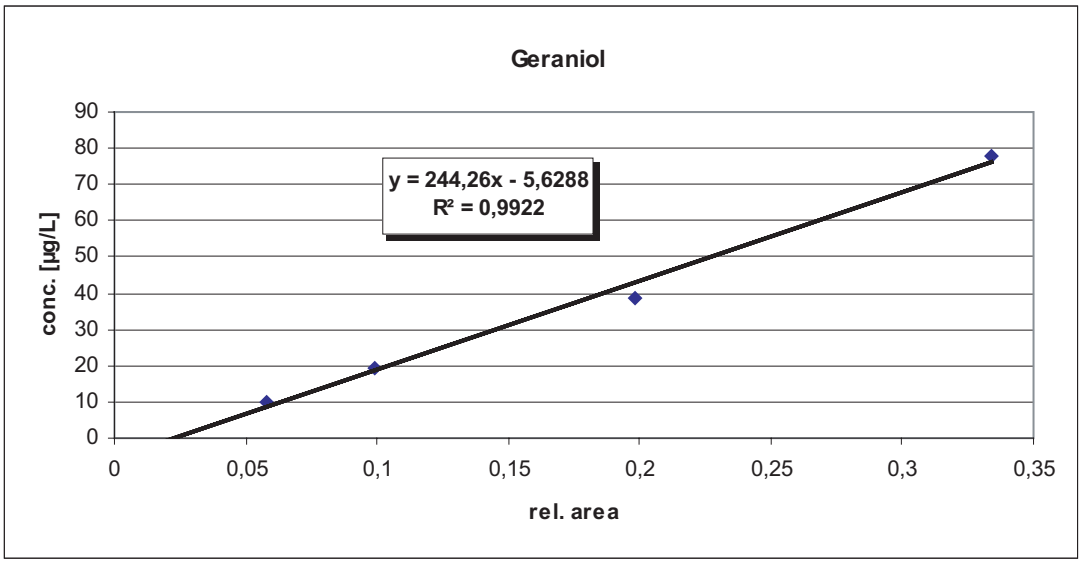

Figure 1 Calibration plot for Geraniol