S. Hanke, M. Herrmann, J. Rückerl, C. Schönberger, W. Back

Hop Volatile Compounds (Part II): Transfer Rates of Hop Compounds from Hop Pellets to Wort and Beer

Several hundred aroma compounds are known in hops but only a few have great impact to hop aroma of beer. Adding hops at late stages of boiling gives a pleasant hoppy flavour to the final beer. Linalool is known to be a good indicator for such a hoppy flavour. In this study it could be shown that different hop varieties have different transfer rates of linalool and other aroma compounds. The behaviour of the aroma compounds is different and compound specific. Linalool increases during fermentation and there were differences between beers fermented at 8 °C and 12 °C. The 12 °C samples showed lower concentrations of linalool but higher scores in aroma intensity. Possible explanation are additive interactions between fermentation-by-products and hop aroma compounds.

Descriptors: hop aroma, linalool, geraniol, hop flavour, hops, Humulus Lupulus L.

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 different processing steps during brewing the hop aroma of beer is very different from the aroma of the hop product [2, 3]. Myrcene, the primary compound of the hop oil could not be found in the final beer [1]. Therefore the fine hoppy flavour of a beer flavoured with hops in the kettle/whirlpool is due to other compounds.

Since a good correlation between the concentration and the perceived hop aroma intensity the terpen alcohol linalool exists it was claimed as character impact compound for the hoppy flavour by *Kaltner and Fritsch* [4–6]. The intensity of hoppy flavour in the final beer depends on different aspects during wort processing. For example different boiling systems and time of hop addition [7, 8] as well as annual variation in hop oil composition [9] could lead to a different hop aroma from one year to another and these changes in product quality will be detected by the consumer. The common way, to create a hoppy flavour is a dosage of hops at late stages of wort boiling or in the whirlpool. This results in a pleasant hop aroma since the evaporation of the hop aroma compounds is minimized [4, 5, 10–12]. Depending on the hop variety used the hop aroma of the final beer can vary in different ways (citrussy, herbal, spicy, flowery, fruity) [5, 13, 14]. In general the last hop dosage is added according to the α acid content of the hops. But since the α-acid content and the aroma content show no correlation at all, the last addition of hops should be calculated according to the aroma content rather than the bitter acid content

Authors: Dipl.-Ing. Stefan Hanke, Dr. Markus Herrmann, Prof. Dr. Werner Back und Johannes Rückerl, Lehrstuhl für Technologie der Brauerei I, Technische Universität München, Center of Life and Food Sciences, Weihenstephaner Steig 20, D-85354 Freising-Weihenstephan, Germany, Stefan.Hanke@wzw.tum.de;
Christina Schönberger, Joh. Barth & Sohn GmbH & Co. KG, Freiligrathstrasse 7/9, D-90482 Nürnberg, Germany

Tables and Figures see Appendix.

2 Material and Methods

Aroma compound analysis was carried out according to a previously published method [15]. The measurement of the hop volatile compounds was carried out by water steam distillation and measurement by GC-FID in accordance to the similar method described by MEBAK [1]. The substances in question were calibrated by an additive 6-point calibration. All other analysis were carried out according due the relevant MEBAK [16] or EBC-methods [17]. Wort was prepared in a 60-L-pilot-brewing-plant. The used mashing regime was 62 °C 20 min, 65 °C 20 min, 72 °C 30 min, 78 °C 1 min. For mash separation a lauter tun was used with two spargings. The wort was boiled without hop addition for 60 minutes without any hop addition and for the trub separation a whirlpool was used. The hop addition was during the transfer of wort from the kettle into the whirlpool. In a previous study it was demonstrated that the content of the indicator substance Linalool was highest when hops are added in the whirlpool [4]. The amount of hops added was calculated using the Linalool content measured in the pellets. Four hop varieties (2006 harvest) were used in this study. The analytical data of the hop samples are shown in Table 1 and the added amount of the different hop varieties are shown in Table 2. Fermentations were carried out in small scale cylindroconical vessels (10 L) at 8 and 12 °C with bottom fermenting pure culture yeast, the pitching rate was 12 million cells per ml. After main fermentation the green beer was maturated 2 days at 16 °C, stored for 2 weeks at 0 °C and finally filtrated and bottled. The beers were tasted according the tasting scheme of Kaltner [18] with special focus on intensity and quality of hoppy flavour.

3 Results and Discussion

Linalool is known to be a good indicator for hoppy flavour. Its threshold in beer is reported to be 5 µg/l [4, 19]. *Meilgaard* reports a threshold of 80 µg/l [20]. Due to its good indicator properties the current paper focus on this substance. In Figure 1 and 2 the transfer rates of linalool and geraniol from hop pellets into wort are presented. Given identical conditions in the whirlpool, we must assume that the transfer rates for linalool depend to some degree on the variety and to a larger degree on the total amount of hops added. Only the variety Spalt Spalter (SSP) has a significantly lower transfer rate within the margin of error of the analysis, whilst the others have to be considered equal. These transfer rates for linalool were found to be in the range as previously published by *Steinhaus et al.* [14]. They reported transfer rates for linalool between 80 and 110 %. For geraniol, another potent hop aroma compound, Steinhaus et al. reported transfer rates between 35 and 91 %. In the current research the transfer rate for geraniol was found to be much higher and in the range between 120 and 240 %. It is unclear where these discrepancies come from. An acid promoted release of glycosidic bound geraniol is unlikely since geranyl-β-glycoside seemed to be stable in a heated acidic model solution [21]. A transformation into other aroma compounds as described by *Baxter et al.* [22] and *Ohta et al.* [21] would be a possible explanation.

The concentration of linalool increased during fermentation (see Fig. 3). Kaltner found also such an increase of linalool [4]. He supposed that glycosidically bound flavours are released during the fermentation and therefore cause an aroma increase. Belgian researchers showed that the β-glucosidase activity is strain dependant and seems in beer-making favourable [23]. The amount of glycosides is variety dependant [24]. Only the beers produced with HOL showed an increase of linalool with an increase in fermentation temperature from 8 to 12 °C. The other three varieties showed a decrease with increasing fermentation temperature. A higher fermentation temperature causes higher fermentation rate and higher production of carbon dioxide. Linalool could be evaporated by the higher CO_2 production.

In Figure 4 the trend of geraniol is shown from wort to beer. In these experiments all varieties showed at the higher concentration levels (level 3) of geraniol a decrease during fermentation. Yeasts are able to biotransform some monoterpene alcohols [25] and hydrogenate geraniol into citronellol [14, 25]. At lower levels (level 1 and 2) an increase of this aroma compound was found. Ohta et al. showed that the glycoside of geraniol was unstable to exposed β-glucosidase activity [21] and geraniol was released from its glycoside. At the lowest and partially at middle levels the concentration of geraniol increased during fermentation and it showed an increase with increasing fermentation temperature. A possible explanation is that a release from glycosides was higher than a possible biotransformation of geraniol.

Beside linalool and geraniol there a further aroma compounds. Since they do not exceed their individual thresholds they only contribute to beer flavour in additional or synergistic ways. As can be seen in Table 3 the behaviour is quite different and compound specific.

The concentration of α-terpineol (threshold in beer: 2000 µg/L [26]; lilac or pine like flavour) increased during the 8 °C fermentation. Beers fermented at 8 °C showed also the higher levels of α-terpineol (15-35 µg/L). The transfer rate of α-terpineol to wort showed great differences between the three dosage levels. At level 1 all four varieties showed transfer rates above 150 %. In contrast at level 3 all varieties showed a transfer rate below 90 %.

β-caryophyllene (threshold in beer: 600 µg/l [2]; spicy, wooden flavour) can be found in all wort samples but only in some beers. A similar behaviour of this substance was found *Mitter et al.* [8]. The transfer rate of this compound did not exceeded 40 % at any addition level and was found to be highest for the variety HOL. Other varieties showed transfer rates of 5 to 20 %. If β-caryophyllene was detected in beer than in all cases the concentration increased from hot wort to final beer.

Nerol (threshold: 500 µg/L [2], lime and rose like flavour), is the cis-isomer of geraniol. At low dosage levels the utilization rate into wort exceeded 200 % and it decreased with higher dosage levels. During fermentation there was found an increase for most experiments. Hardly any difference was found for different fermentation temperature. The concentration of nerol never exceeded 10 µg/l.

α-Humulene (threshold: 800 µg/L [2], carnation like flavour) showed a indifferent behaviour. With increasing hop addition the levels of α-Humulene increased, as well. Mitter et al. found that α-Humulene was present in small amounts in wort but it disappeared during fermentation [8]. In the present study α-Humulene was present in all worts (10–40 µg/l) and for some beers the concentration of α-Humulene increased during fermentation.

The sensory analysis of the beers was done according the tasting scheme of Kaltner presented elsewhere [18]. Tasters were advised to evaluate the intensity and quality of the hop aroma in taste and odour. The graph in Figure 5 shows the score for aroma intensity in odour versus the linalool content in all final beers. This increasing score in intensity with increasing linalool concentration confirmed the findings of previous studies [5, 10].

A comparison of the beers fermented at different temperature levels showed that the aroma intensity and quality increased with the higher temperature. In Figure 6 the data for the variety HTU are presented and the other varieties showed similar results. The beers were made from the same wort but the beers fermented at 12 °C showed lower linalool concentrations than the 8 °C beers. The fact that with lower linalool levels higher aroma intensities were found could be with additional effects of the hop aroma compounds and fermentation-by-products like 2-phenylethanol, 2- and 3-butylacetate (fruity flavour). For example 2-phenylethanol has a rose-like, flowery flavour and it is possible that there are additive interactions between other flowery hop derived flavours (geraniol, nerol). Such additive effects of aroma components of one organic class are well-known in flavour chemistry [19, 27]. But it is also possible that compounds of different chemical classes with a similar flavour impression could interact and contribute together to a e.g. rose-like or flowery flavour. But it is necessary to study the temperature effect for a broader temperature range as well as for different yeast strains.

The low content of 1 to 5 mg/l iso-α-acids in the beers stems from post-isomerization in the whirlpool; the worts have not been hopped during boiling to eliminate possible influences on the results from residual remains of hop volatile compounds. The average utilization rate of the bitter acids was 5.4 %. Unisomerized α-acids were also in small amounts detected. The concentration varied from 0 to 5 mg/l, beers with the highest HTU dosage showed up to 7 mg/l humulones.

4 Summary

By using a linalool based late hopping procedure it is possible to achieve constant aroma quality. Compared to a bitter acid based hopping procedure the linalool based dosage is more practical especially since the concentration of aroma compounds is subject to seasonal or variety derived changes. The transfer rate of aroma compounds has to be considered equal between three of the tested varieties, with only Spalt Spalter (SSP) showing an analytically significant difference. This might be a hint on an influence by the growing conditions (climatic conditions) and should be considered in further studies. If hops for the late hop dosage will be replaced the transfer rate for the new batch should be determined again. At different hopping levels the transfer rate changes showing a decrease with increasing hop addition.

The sensory analysis in this study showed that linalool is a suitable indicator for the hop aroma and a linalool based dosage should be realised to achieve a constant hop aroma.

In the future the influence of fermentation temperature will be a focus of research since it was found that beers fermented at higher temperatures showed a better quality and a higher intensity of the hoppy flavour.

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Appendix

Table 1 Analytical data of the Hop varieties

	Hallertau Taurus HTU	Hallertau Opal HOL	Spalt Spalter SSP	Hallertau Saphier HSR
µg/g hop				
Geraniol	11.0	11.0	13.0	4.1
α-terpineol	12.0	5.4	5.3	3.8
β-caryophylene	60.0	15.0	38.0	14.0
Nerol	2.7	1.5	2.0	0.7
α-Humulene	134.0	35.0	93.0	32.0
Linalool	103.0	77.0	50.0	29.0
LCV EBC 7.5 [%]	14.6	5.7	5.0	3.2
α-acids EBC 7.7 [%]	13.1	4.9	4.1	2.3
Linalool/EBC 7.5	7.1	13.5	10.0	9.1
Linalool/EBC 7.7	7.9	15.7	12.2	12.6

Table 2 Dosed amount of Hop pellets, α-acids and Linalool per litre

hop variety	HOL			SSP		
	g hops/L	µg Linalool/L	mg α/l	g hops/L	µg Linalool/L	mg α/l
Level 1	0.37	28	18	0.40	20	16
Level 2	0.74	57	36	0.80	40	33
Level 3	1.47	113	72	1.60	80	66
hop variety	**HTU**			**HSR**		
	g hops/L	µg Linalool/L	mg α/l	g	µg hops/L	mg α/l Linalool/L
Level 1	0.19	20	25	0.69	20	16
Level 2	0.39	40	51	1.38	40	32
Level 3	0.77	80	101	2.76	80	63

Table 3 Hop aroma compounds of the different worts and beers

	geraniol			α-terpineol			β-caryophylene		
	wort	*8 °C*	*12 °C beer*	*wort beer*	*8 °C*	*12 °C beer*	*wort beer*	*8 °C beer*	*12°C beer*
HOL 1	7	11	14	4	22	tr.	2	tr.	tr.
HOL 2	11	13	19	5	20	4	3	6	3
HOL 3	20	11	24	7	23	7	5	3	14
HSR 1	6	16	16	4	26	5	2	tr.	4
HSR 2	9	13	16	5	14	5	2	3	5
HSR 3	15	14	16	7	34	6	3	4	5
HTU 1	5	13	12	3	29	4	2	3	tr.
HTU 2	9	16	15	4	29	5	2	tr.	tr.
HTU 3	17	14	15	7	29	3	5	tr.	tr.
SSP 1	10	13	15	4	18	9	1	10	tr.
SSP 2	19	14	16	6	17	4	2	0	tr.
SSP 3	35	18	22	7	23	5	4	5	10

	nerol			α-humulen			linalool		
	wort	*8°C beer*	*12°C beer*	*wort*	*8°C beer*	*12°C beer*	*wort*	*8°C beer*	*12°C beer*
HOL 1	1	4	5	18	14	92	22	29	30
HOL 2	2	5	4	24	34	27	39	60	63
HOL 3	3	7	7	33	22	34	70	102	147
HSR 1	1	4	5	18	13	43	21	38	33
HSR 2	1	5	5	18	7	11	34	58	59
HSR 3	2	6	6	24	9	8	61	106	93
HTU 1	1	5	4	20	10	9	17	30	26
HTU 2	2	5	5	25	8	12	32	58	53
HTU 3	4	4	3	41	21	17	62	95	91
SSP 1	2	6	9	10	34	33	11	18	17
SSP 2	2	5	4	16	38	29	21	27	24
SSP 3	3	5	5	34	29	33	41	53	53

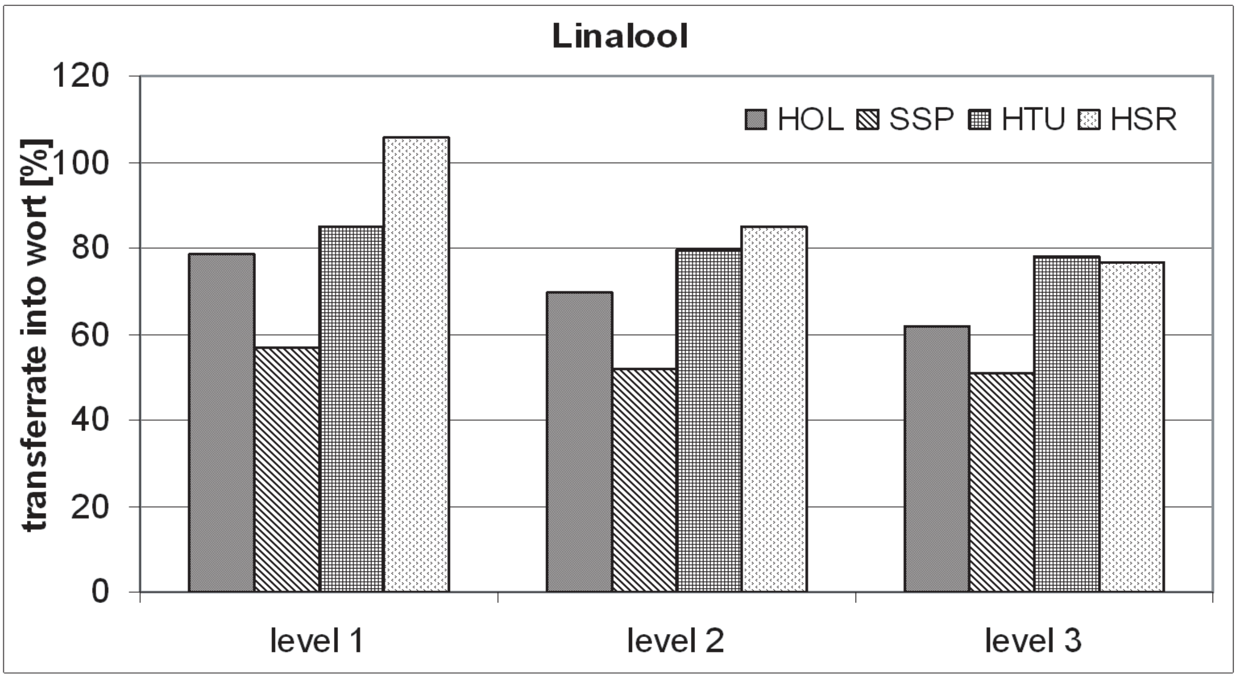

Figure 1 Transfer rate of Linalool into Wort

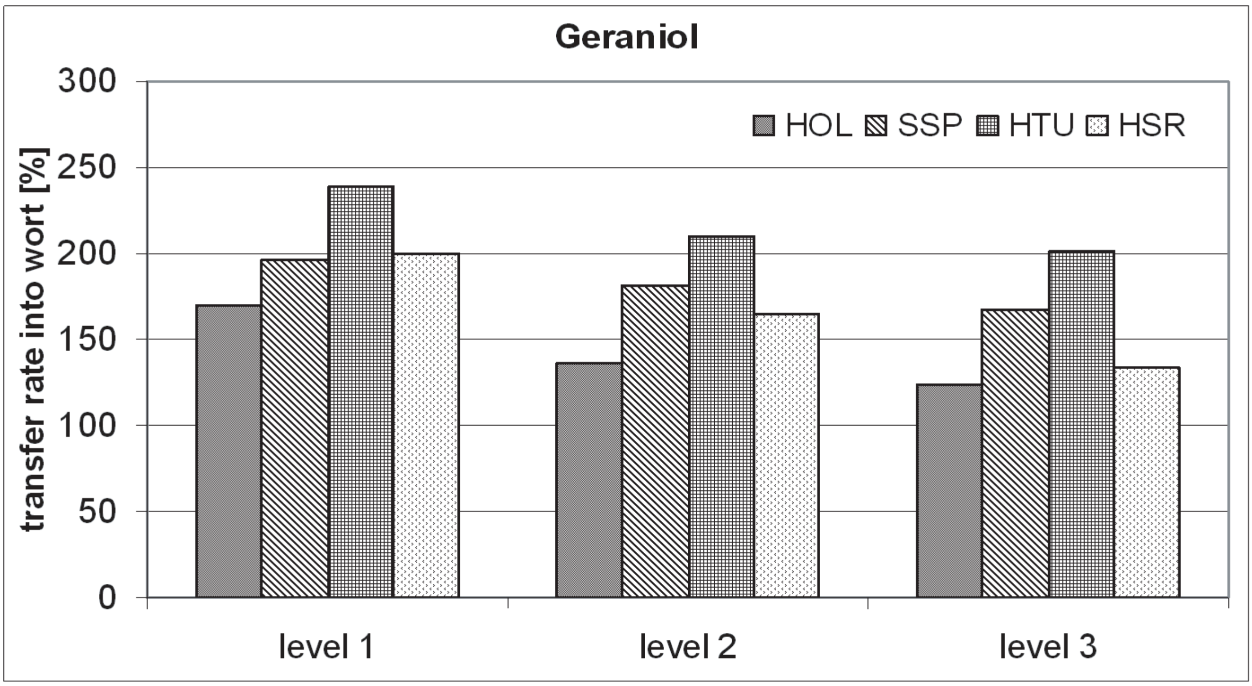

Figure 2 Transfer rate of Geraniol into Wort

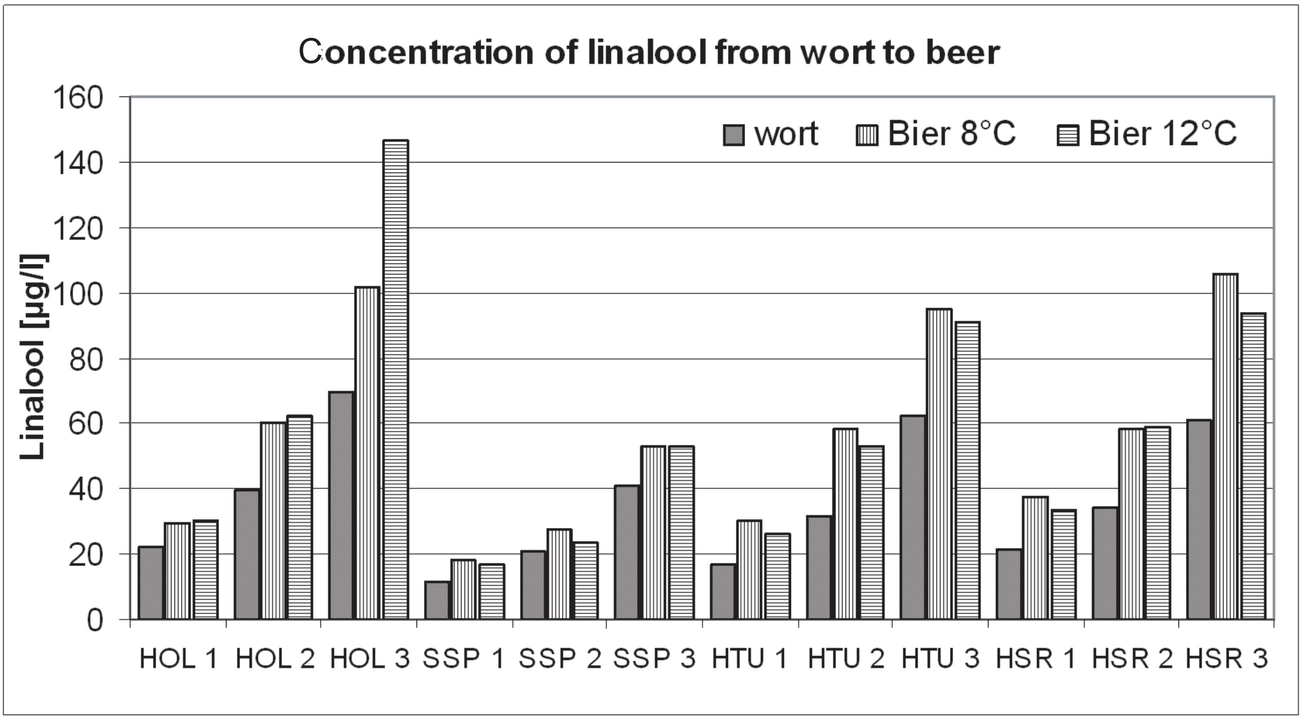

Figure 3 **Concentration of Linalool from Wort to Beer**

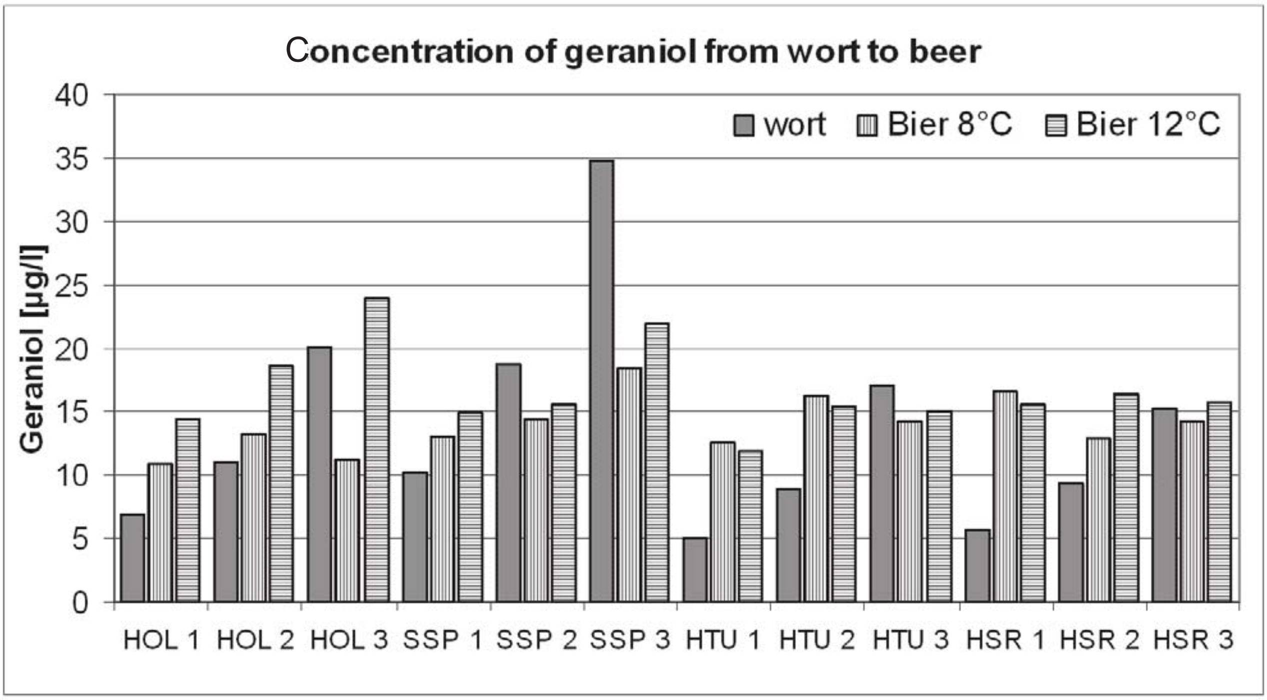

Figure 4 **Tration of Geraniol from Wort to Beer**

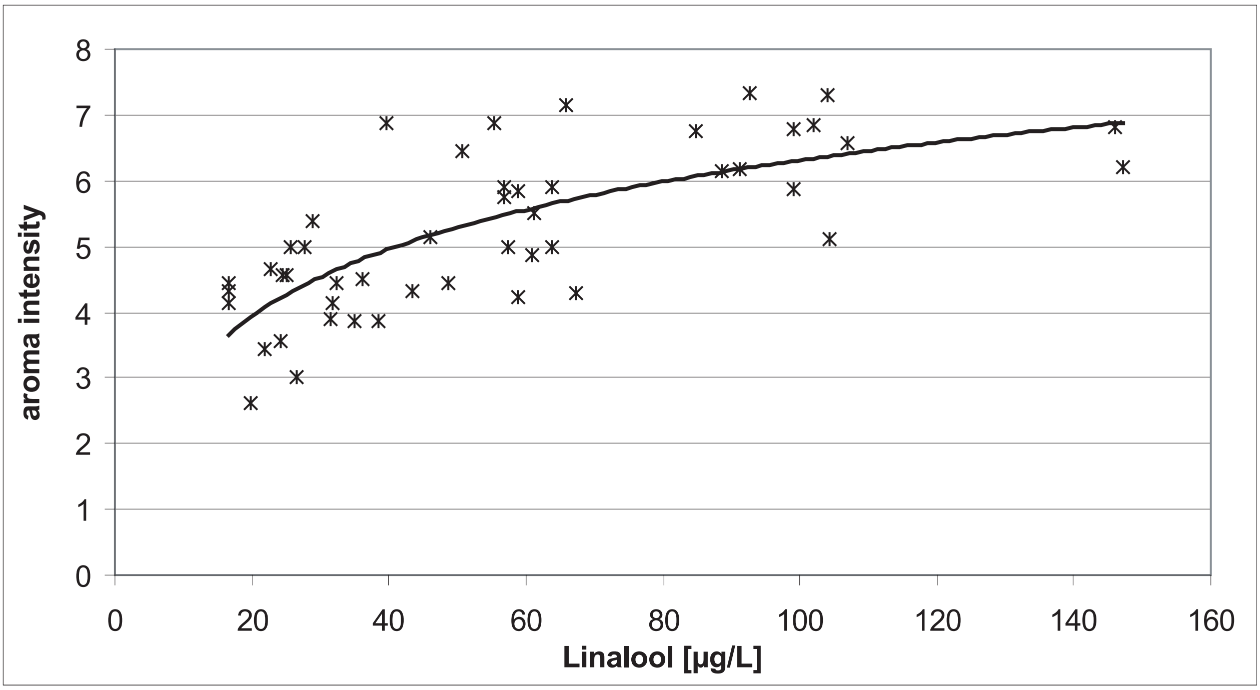

Figure 5 Influence of Linalool to Aroma Intensity

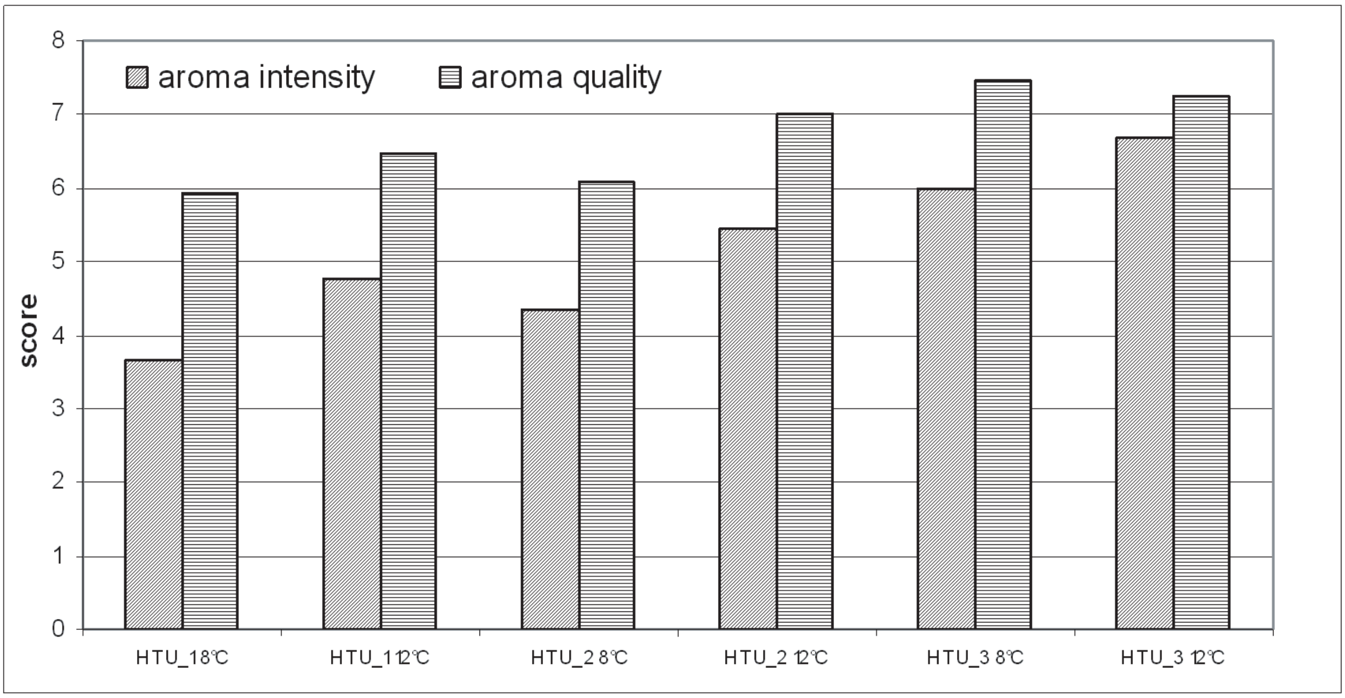

Figure 6 Aroma Comparison of 8 °C and 12 °C fermented Beers