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On the Fate of Certain Hop Substances during Dry Hopping

Dry hopping is becoming increasingly popular especially in small breweries. It is a complex and sophisticated method, but it is exactly those qualities which make it a highly efficient method for craft brewers to stand out among the mass of other beers. Empirical experience is the key factor here in the choice of hops and type of application. There is still little known about the transfer rates of hop substances during dry hopping which can provide a great variability of application.

A test was made in which four dry hopped pale lager beers were contrasted with a similar produced beer without dry hopping. Here the new German varieties *Mandarina Bavaria*, *Hüll Melon*, *Hallertauer Blanc* and *Polaris* were used for dry hopping. The dosed quantity of 1.5 ml/hl was based on the hop oil content. The transfer rates were calculated from the difference between analysis values of the dry hopped beers and the control beer divided by the dosed dry hopping quantities. As the calculations were made from three analytical values they inevitably produced relatively large ranges of fluctuation.

Of the dosed α -acids, 4 to 5 % can be found in the beers, of the total polyphenols 50 to 60 % and of the low-molecular polyphenols 60 to 70 %. The transfer rates of individual polyphenols show systematic differences; there is no recognizable dependence on variety. The behaviour of the aroma components examined is also not uniform. Terpene hydrocarbons show low yields of about 3 %; linalool transfers to about 100 %. Geraniol seems to react variety-specific with two yields of approx. 50 % and two significantly over 100 %. There is also a variety-specific phenomenon with 2- and 3-methylbutyl-2-propanoate. Chemical transformations with or without yeast enzymes are probably the cause. The results show just how much more work has to be done.

The reductions in and transformations of hop aroma substances during ageing of beers are an indication that intensity and type of aroma are subject to changes.

The five beers were tasted by a consumer panel of 30. The dry hopped beers had a very intense hop aroma and also a surprisingly intense body. The quality of the hop aroma prevailed over personal preferences and the quality of bitterness was appraised the same.

Descriptors: dry hopping, transfer rates, hop aroma compounds, polyphenols

1 Introduction

Dry hopping is a technology that is drawing increasing attention. Normally whole hop cones, ground hops or pellets are added to the cold beer to transfer in particular aroma components to the beer with low losses (no evaporation) and reduced chemical transformations (no thermal load, less influence of yeast) [1, pages 295–301, 2, 3, 4, 5, 6].

There are a few statements available on the technology and technique of dry hopping [1, 2, 6, 7]. *Grinnell* [7] emphasizes the particular difficulties of a reproducible transfer of hop substances with dry hopping. Hop aroma stability is also a topic in many discussions among brewers [2, 8, 9]. Hieronymus reports on the stability of a dry hop aroma as explained to him by craft brewers [2,

pages 234–238]. Typical are statements that indicate variety-dependent changes. The stability of a dry hop aroma often differs from a late hop aroma despite using the same hop variety [9]. Possible aroma changes are the consequence of numerous phenomena such as

- Adsorption to crown caps (depending on substance) [10]
- Chemical reactions (e.g. oxidation)
- Reactions with beer ingredients (e.g. ethanol)
- Enzymatic reactions

It is obvious that the type and intensity of these processes depend on the type of beer, packaging, composition of the hop aroma components and thus the hop variety and application technology (e.g. time, temperature, solution technique, yeast strain and yeast cell count) as well as the storage conditions.

It is interesting to note here that the plastic liner in crown caps can absorb terpenes almost completely from the beer [10].

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The information about the transfer of hop substances spreads widely. In [6], it was established that dry hopping led to an increase in linalool exceeding the threshold value. An increase in some polyphenols and the total polyphenols was also observed. In model trials, transfer rates from hops to beer of approx. 80 % for linalool and geraniol were found and from 0.3 to 2.6 % for myrcene, β -caryophyllene and α -humulene [11]. Depending on the hop variety, the values of myrcene rose to approx. 100 ppb, of linalool to 80 to 180 ppb, of geraniol to 5 to 30 ppb and of α -terpineol to 15 ppb. These tests were carried out in 30 liter kegs [12]. In the same trials, slight increases in polyphenols and anthocyanogens were determined in some but not all of the varieties used.

Whereas in the trials already mentioned it was found that the content of the aroma components examined rose with the increase of the hop addition, this was contradicted in another test [13]. Doubling the hop additions from 200 to 400 g/hl did not lead to an increase of myrcene, linalool, geraniol, β -caryophyllene and α -humulene, which was reflected also in the tasting results.

In [4], transfer rates of about 6 % for the α -acids were mentioned and less than 1 % for myrcene. Depending on the dosing method, the increases in linalool achieved can be considerable, but the transfer rates are not specified.

Since information about transfer rates in particular is rather sparse, this question was addressed in an indicative series. Also, the behaviour of some hop aroma components was analysed in the ageing process of beer.

2 Test Program

Five beers were brewed in the St. Johann research brewery (2 hl). The basis and comparison was a hoppy pale lager with the following characteristics:

- Original gravity 11.5–12.0 %.
- Bitterness approx. 25 IBU.
- Four-stage hopping with normal Type 90 pellets of *Hallertau Tradition* (HHT) and *Hallertau Mittelfrueh* (HHA).
- Dose of 75 g/hl HHT at the beginning of boiling.
- Dose of 70 g/hl HHT in the middle of boiling.
- Dose of 50 g/hl HHT at the end of boiling.
- Dose of 50 g/hl HHA into the whirlpool.
- Boiling time (internal calandria) 75 minutes.
- Cold main fermentation approx. 1 week at 9 °C, maturation approx. 1 week at 15 °C, cold storage 3 weeks at 0–1 °C.

The four recently approved new cultivars from Huell with special aromas *Mandarina Bavaria* (HMB), *Huell Melon* (HHN), *Hallertauer*

Blanc (HHC) and *Polaris* (HPA) were used for dry hopping in the form of ground hop cones. Characterizations are available in the references [14, 15]. The amounts of hops in each case were added into the empty storage tank, the tank flushed with CO₂ and the green beer was pumped into the storage tank after main fermentation. The hops remained in the tank during maturation and storage and were purged together with the yeast the day before filtration.

It has been reported [4] that the substance transformation from hop cones and pellets is not identical. In particular pelleting crushes the membrane of the lupulin glands, which is useful for the solubility of aroma substances. Since in the present case not all varieties were available in pellet form, ground hops were used throughout.

Table 1 shows the doses in g per hl. They are calculated from the hop oil content (EBC 7.10) and the desired dose rate of 1.5 ml hop oil per hl.

Table 1 Dry hopping doses for 1.5 ml hop oil/hl

Variety	Abbrev.	Oil (ml/100g)	Dosage (g/hl)
Mandarina Bavaria	HMB	2,05	73
Hüll Melon	HMN	1,45	103
Hallertauer Blanc	HHC	1,65	91
Polaris	HPA	3,80	40

3 Hop Analyses

Table 2 shows the analysis results of the samples used for dry hopping.

Table 2 General analysis data of hops used for dry hopping

Feature	Unit	HMB	HHN	HHC	HPA
α -acids (EBC 7.7)	% w/w	8,7	7,0	9,8	19,5
β : α (EBC 7.7)	–	0,76	1,22	0,56	0,26
Cohumulone (EBC 7.7)	% rel.	31	28	22	25
Total polyphenols (AHA)	% w/w	4,4	4,3	5,6	3,8
Polyphenols : α	–	0,51	0,41	0,57	0,17
HPLC-polyphenols	% w/w	1,10	1,17	1,36	0,66
HPLC-PP:total PP	% rel.	25	27	24	17
Total oil (EBC 7.10)	ml/100g	2,05	1,45	1,65	3,80

The α - and β -acids are to be understood as values according to EBC 7.7. The total polyphenols were determined according to a non-specific AHA method (AHA = Arbeitsgruppe Hopfenanalyse = Hop Analysis Working Group). This method is based on a hot water extraction of hops or hop products. After filtration of the aqueous solution a spectrophotometric determination of the polyphenols according to EBC 9.11 is carried out. A HPLC method provides the low-molecular polyphenols and is described in [16]. The sample is extracted with acetone/water and cleaned by washing with hexane. The polyphenols are adsorbed on polyamid, released with a methanol washing and separated and quantified by gradient HPLC

Table 3 Contents of 11 low-molecular polyphenols in mg/100 g; four new varieties compared with a *Hallertauer Mittelfrueh*

	mg/100g				
	HHA	HMB	HMN	HHC	HPA
Procyanidine C/C/C	103	20	20	22	8
Procyanidine B3 C/C	154	56	69	64	22
Procyanidine B1 EC/C	206	74	72	94	27
Catechine	287	51	89	76	33
Epicatechine	53	44	69	62	31
Coffeoylquinic acid	96	68	69	59	29
Feruoylquinic acid	63	21	21	18	8
Quercetin glucoside	158	87	153	178	67
Quercetin malonyl hexoside	310	132	83	164	56
Kaempferol-3-glucoside	75	42	95	107	50
Kaempferol malonyl hexoside	235	91	69	151	78
Total	1740	686	809	995	409

combined with a Diode Array Detector. From the approximately 60 determined substances the most interesting are selected.

The four varieties can only be classed conditionally as aroma hops. Both the α -acid content and the polyphenolic data deviate from the norm, particularly in the case of *Polaris*. With 20 % α -acids, only 3.8 % total polyphenols and a low content of low-molecular polyphenols and β -acids, it corresponds to a typical high alpha variety.

Table 4 Contents of 20 selected aroma compounds in mg/100g; four new varieties compared with a *Hallertauer Mittelfrueh*

	HHA	HMB	HMN	HHC	HPA
Myrcene	173	833	771	914	2248
Ocimene	1	9	14	5	107
β -Caryophyllene	77	46	25	52	278
Farnesene	4	17	132	4	3
α -Humulene	273	143	25	145	739
β -Selinene	4	54	97	103	11
α -Selinene	6	59	96	115	16
Selina-(4,7) + (3,7)-diene	2	0	27	1	1
Isobutyl isobutyrate	1	12	14	6	28
Isoamyl propanoate	1	5	5	10	28
3-Methylbutyl-2-propanoate	1	4	8	6	19
2-Methylbutyl-2-propanoate	4	30	48	26	89
Methyl heptanoate	1	12	7	6	17
Methyl octanoate	1	11	8	4	66
Octyl isobutanoate	2	3	3	3	61
Methyl-4,8-decadienoate	4	9	12	10	34
Geranyl acetate	0	1	1	8	17
Linalool	6	6	4	7	9
Geraniol	1	14	8	2	4
Epi-Cubanol	3	4	4	7	6

Table 3 shows 11 low-molecular polyphenols with their contents in mg/100 g compared with a typical *Hallertauer Mittelfrueh*. The quantities vary according to variety over a larger range as known from other papers [18].

The aroma substances of the hops were quantified using GC-FID [17] after separation of a distilled oil in a hydrocarbon and an oxygen fraction. This analysis is different to EBC 7.12, where the total hop oil gained with the EBC 7.10 method is used directly for GC analysis. 20 possibly interesting single substances are listed in table 4. Linalool

and epi-Cubanol have the least spread ocimene has the highest. The new varieties showed a number of specific characteristics:

- Ocimene extraordinarily high in *Polaris*.
- α -humulene extremely low in *Huell Melon*.
- α - and β -selinene unusually high in *Huell Melon* and *Hallertauer Blanc*.

■ The sum of Selina-(4,7) and Selina-(3,7)-diene only very high in *Huell Melon*.

■ All four hops, but in particular *Polaris*, have unusually high ester contents.

■ Geraniol is prominent in *Mandarina Bavaria* and *Huell Melon*.

Remarkably linalool, used as one of the primary markers for hop aroma in beer [19], plays the least variety dependent role. This is a clear indication that in the four new varieties and as a difference to established varieties it appears less suitable as a parameter for dosing.

4 Beer Analyses

The reference beer that can already be considered as

Table 5 General analysis data of five beers

	Unit	Control	HMB	HHN	HHC	HPA
Original gravity	Gew.-%	11,58	11,61	11,43	11,77	11,84
Alcohol by volume	%	5,01	5,32	5,18	5,23	5,31
pH		4,47	4,46	4,45	4,51	4,56
Bitterness (EBC 9.8)	IBU	26	27	29	28	27
Iso- α -acids (EBC 9.47)	mg/l	22,8	21,6	21,8	21,5	20,5
α -acids (EBC 9.47)	mg/l	2,8	5,8	5,7	6,4	6,6
Xanthohumol (EBC 9.47)	mg/l	0,0	0,0	0,0	0,0	0,0
Iso-Xanthohumol (EBC 9.47)	mg/l	0,9	0,7	0,7	0,6	0,6
Total polyphenols (EBC 9.11)	mg/l	201	221	219	227	215

Table 6 Contents of a few selected low-molecular polyphenols in the five beers; values in mg/l

	Control	HMB	HHN	HHC	HPA
Procyanidine B3 C/C	1,1	1,4	1,7	1,7	1,3
Catechine	6,4	6,9	7,3	7,0	6,7
Coffeoylquinic acid	1,2	1,8	1,9	1,7	1,4
Quercetin glucoside	2,4	3,1	3,9	3,7	2,7
Quercetin malonyl hexoside	1,2	1,8	1,8	2,0	1,3
Kaempferol-3-glucoside	1,2	1,6	2,2	2,0	1,4
Kaempferol malonyl hexoside	0,8	1,3	1,4	1,6	1,0

Table 7 Analysis results from 12 hop aroma components in the five beers; values in μ g/l

	Control	HMB	HHN	HHC	HPA	Threshold Values	
						[1]	[29]
Linalool	38	87	79	103	76	2 bis 80	2,2
Geraniol	4	54	44	36	42	4 bis 300	29
Myrcene	2,7	10,6	9,3	26,3	21,0	9 bis 1000	
Ocimene	0,4	2,3	2,0	4,7	4,7		
β -Caryophyllene	1,6	1,8	1,6	1,6	1,9	160 bis 420	
α -Humulene	4,0	4,2	4,5	5,6	8,4	747	
Isoamyl propanoate	3,0	25,5	43,5	751	89,4		
3-Methylbutyl-2-methylpropanoate	1,7	25,5	67,5	42,8	76,4		
2-Methylbutyl-2-methylpropanoate	20	338	706	371	596		
Isobutyl isobutyrate	2,2	63,2	86,5	23,8	60,6		
2-Undecanone	0,9	9,0	15,8	23,8	28,8		
Cubanol	2,1	3,8	4,2	3,1	5,2		

complexly hopped and the four dry hopped beers were analysed comprehensively. The EBC 9.47 HPLC method describes only the analysis of iso- α and reduced iso- α acids but delivers also information on α and β acids as well as on xanthohumol and isoxanthohumol when calibrated. Table 5 shows the results.

The deviations of the original gravities, alcohol contents, pH values and iso- α -acids show a good reproducibility of the five brews. An

increase of the pH by dry hopping cannot be derived. The bitterness units rise moderately compared with the reference beer. The α -acid content is significantly higher with a plus on average of 3 mg/l, isoxanthohumol remains constant. As with the α -acids, there is no appreciable isomerization of xanthohumol in the cold. A solution of xanthohumol similar to the α -acids cannot be excluded, but cannot be proven either due to high standard deviations.

Total polyphenols were determined according to EBC 9.11, the HPLC method is based on [16]. Striking is the increase in both total and low-molecular polyphenols, some of which are listed in table 6 for the five beers. An increase is easily recognizable compared with the reference beer.

Finally, table 7 shows the measured values of aroma components in the five beers. The analyses were done at the KAHO (Catholic University) Sint-Lieven, Gent, using a Head-space SPME-GC-MS [20, 21]. Corresponding – partly significant – increases in the dry hopped beers are noticeable immediately. As expected the threshold value of linalool was exceeded significantly already in the reference beer. In the case of geraniol, this was only achieved by dry hopping. Myrcene and some esters have also exceeded their threshold value. There is only few information on the threshold values of hop derived esters in beers. Some figures are shown in [1, pages 201–205]. The values range from 1 to 164 μ g/l. Further data on threshold values of fruity esters (0.3 to 2245 μ g/l) are reported in [22]. Based on this it can be assumed that the threshold values of typical hop esters (see Table 7) lie in a similar range.

The table shows only a few of the main aroma substances of the beers. In addition, no increase in humulene or epoxides could be proven. Analytical data for nerol, citronellol and methyl geranate would be also interesting but were not available.

5 Transfer rates by dry hopping

The example of the α -acids explains the determination of transfer rates with dry hopping (Table 8). The dose of a substance can be calculated in mg/l or μ g/l from the content of a component in the hops and its addition. The value of the reference beer is subtracted from the analysis value of this substance in the dry hopped beers, from which the additional quantity is thus calculated. The additional

Table 8 Transfer rates of the α -acids using dry hopping; systematics of determination

	Unit	Control	HMB	HHN	HHC	HPA
Hop dosage (dry hopping)	g/hl	–	73	103	91	40
α -acids in hops	% w/w	–	8,7	7,0	9,8	19,5
α -dosage (dry hopping)	mg/l	–	63,5	72,1	89,2	78,0
α in beer	mg/l	2,8	5,8	5,7	6,4	6,6
Additional α (dry hopped minus control)	mg/l	–	3,0	2,9	3,6	3,8
Transfer rate = Additional α : Dosage of α	% rel.		4,7	4,0	4,0	4,9

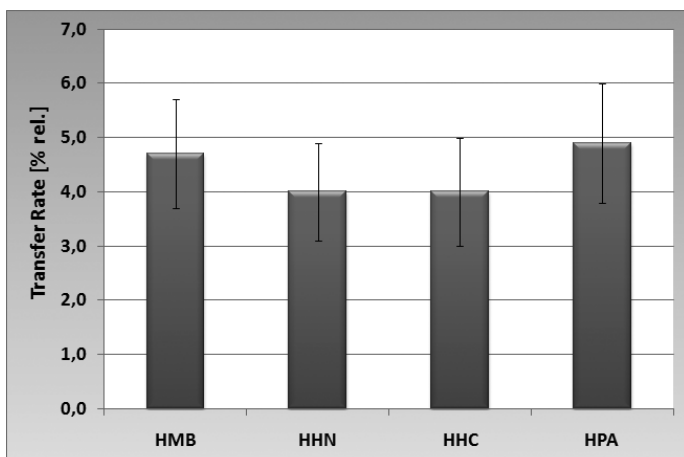
Table 9 Maximum range of variation of the transfer rates in % relative

Substance	HMB	HHN	HHC	HPA
α -acids	1,0	0,9	1,0	1,1
Total Polyphenols	21	17	22	38
Sum of low mol. Polyphenols	28	26	24	56
Procyanidin B3	31	34	32	124
Coffeoylquinic acid	26	28	23	52
Quercetin glucoside	18	20	17	24
Quercetin malonyl hexoside	18	16	14	20
Kaempferol-3-glucoside	28	22	26	58
Kaempferol malonyl hexoside	26	24	22	69
Linalool	16	16	20	21
Geraniol	16	16	21	17
3-Methylbutyl-2-methylpropanoate	26	19	15	27
2-Methylbutyl-2-methylpropanoate	17	18	10	17
Isobutyl isobutyrate	23	15	17	11
2-Undecanon	26	17	21	8

quantity is divided by the dosed amount and in this way the transfer rate is determined in % relative.

In determining transfer rates there is the problem that three analysis values are needed for them:

- The content of a substance in the hops.

**Fig. 1** Transfer rates of the α -acids using dry hopping in % relative

- The content of a substance in the dry hopped test beer.
- The content of a substance in the reference beer, which is subtracted from the test beer.

The transfer rates of the α -acids spread only a little from 4.0 to 4.9 %. Error analysis must however take into account a range of variation of ± 1.0 %. In the present case therefore it can be concluded that the transfer rates do not differ significantly.

Figure 1 shows the relevant exploitation of the α -acids with deviation

The results of the present test program show the same transfer rates of 4 to 5 % of the α -acids. At first this appears low, but is a result of the comparatively high dose of about 3 to 4 mg/l, which is more than double compared with the non-dry hopped reference beer.

Non-isomerized α -acids in the beer are imputed to have a positive foam effect and might also improve flavour stability via chelation of iron [23, 24, 25, 1, pages 249 and 257–259].

The range of variation of the transfer rates is caused by the errors of analysis of the individual determinations in the beers and in the hops. All determinations were made as three-fold analyses. The resulting standard

deviations can be added when calculating additional doses (value in the dry hopped beer minus reference beer). In the worst case the errors of the two beer analyses and of the hop analysis total up here. So the maximum possible deviation of the transfer rate was calculated as follows:

- Maximum additional quantity in the beer:dosed minimum quantity = maximum transfer rate in % rel.
- Minimum additional quantity in the beer:dosed maximum quantity = minimum transfer rate in % rel.

The maximum ranges of variation were determined this way and are given in table 9 and shown in figures 1 to 4 in the form of error bars.

The procedure described in table 8 for determining transfer rates can be applied for all substances for which analysis data in hops and beer are available. The corresponding data for the four hops is given in tables 2 to 4 and in tables 5 to 7 for the five beers. Table 10 summarises the transfer rates of the polyphenols and provides information for the total polyphenols, the sum of low-molecular polyphenols and 7 single substances.

Figure 2 shows the transfer rates of the total polyphenols and individual polyphenols with ranges of variation. No significant

Table 10 Transfer rates of total polyphenols, the sum of low-molecular polyphenols and 7 single substances; specifications in % relative

Substance/Group	HMB	HHN	HHC	HPA	Ø without HPA
Total polyphenols	62	41	51	90	52
HPLC-Polyphenols	86	67	49	92	77
Procyanidine B3 C/C	73	85	103	222	79
Catechines	135	99	87	308	117
Coffeoylquinic acid	120	99	94	167	110
Quercetin glucoside	117	94	81	100	106
Quercetin malonyl hexoside	63	71	54	45	67
Kaempferol-3-glucoside	133	100	80	100	117
Kaempferol malonyl hexoside	76	85	58	65	81

The transfer rates of four single components with the corresponding maximal fluctuations are shown in figure 3.

The good solubility of some low-molecular polyphenols can have the following effects:

- Proanthocyanidins as complexes with middle- and high-molecular proteins can increase the tendency of a beer to form a haze.

Table 11 Transfer rates of some polyphenols in comparison; hop dosing in the brewhouse and dry hopping

Substance	Transfer rates % rel.	
	Wort	Dry Hopping
Total polyphenols	50 to 70	50 bis 60
Procyanidine B3 C/C	30	ca. 100
Catechine	66	ca. 100
Coffeoylquinic acid	118	> 100
Quercetin glucoside	96	100
Quercetin malonyl hexoside	60	58
Kaempferol-3-glucoside	100	103
Kaempferol malonyl hexoside	43	71

- Non-oxidized polyphenols positively influence the body (mouth feel) of beers [1, p. 290, 18]

- Polyphenols are said to improve the flavour stability of the beer [1, p. 291]

In this context it is of interest to make a comparison with the scarce information given in the literature [1, pages 285–287, 18]. For example, a number of transfer rates of polyphenols are specified with dosing in the hot wort in the middle and at the end of boiling. Table 11 shows a comparison of “hot” and “cold” transfer rates.

The haze active substances procyanidin B3 and catechin suffer tangible losses through precipitation in the wort, but not in the beer. In contrast, the transfer rates of the total polyphenols and the 5 other components show a similarity. In particular the differences in the glucosides (approx. 100 %) and malonyl hexosides (43 to 71 %) match. The enzymatic potential of the yeast may play a role here.

differences between the varieties can be stated. However, the following can be established:

- The yield of total polyphenols is 50 to 60 % despite low pH and low temperature of the beers.
- Low-molecular polyphenols show a slightly higher yield.
- Procyanidin B3, catechin, coffeoylquinic acid and both glucosides transfer almost completely, considerably lower on the other hand are quercetin malonyl hexoside and kaempferol malonyl hexoside.

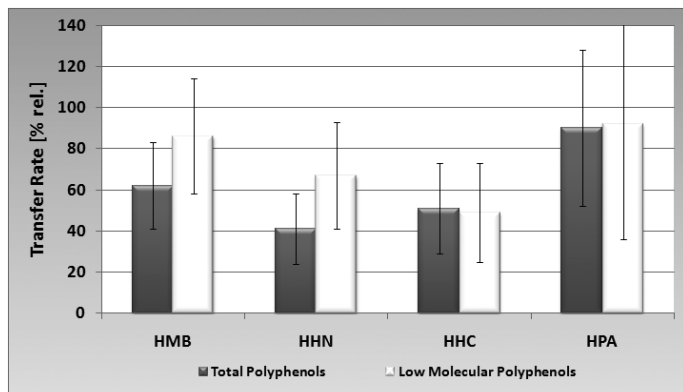


Fig. 2 Transfer rates of the total polyphenols and the low-molecular polyphenols in %

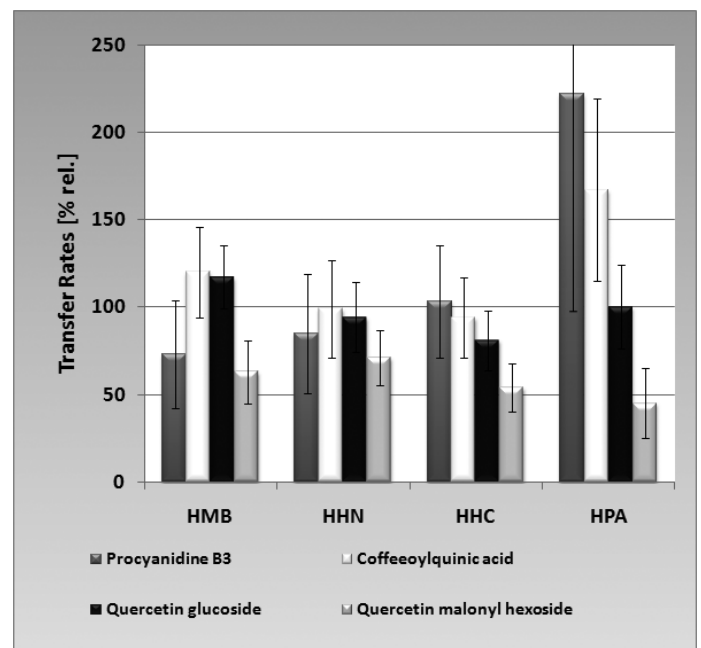


Fig. 3 Transfer rates of 4 single components of the polyphenols in relative %

Table 12 Transfer rates of some aroma substances by dry hopping (in % relative) from the hops into the beer

Substance	HMB	HHN	HHC	HPA
Linalool	111	100	102	106
Geraniol	49	49	178	138
Myrcene	0,1	0,1	0,3	0,2
Ocimene	2,9	1,1	9,3	10
β -Caryophyllene	0,6	–	–	0,02
α -Humulene	1,9	0,2	0,1	0,2
Isoamyl propanoate	70	79	106	77
3-Methylbutyl-2-methylpropanoate	40	161	75	99
2-Methylbutyl-2-methylpropanoate	62	222	148	162
Isobutyl isobutyrate	70	58	40	52
2-Undecanone	37	34	84	58
Cubanol	6	5	2	13

The transfer rates of 11 selected hop aroma substances were determined the same way and are shown in table 12.

Figures 4 to 6 show the percentage transfer rates for six single substances.

Some knowledge can already be derived from these data and diagrams:

- The linalool yield is about 100 %. Whether yeast can release glycosidically bound linalool cannot be proven.
- With geraniol things are not clear. Two varieties show a transfer rate of about 50 % and two others significantly over 100 %. An eye should be kept on this phenomenon, because there are indications that precursors of geraniol lead to different contents via enzymatic activities of yeast [26]. It might also be possible that – depending on hop variety – glycosidically bound terpene alcohols exist in varying quantities and can be separated by yeast enzymes. With this, a source of terpene alcohols like geraniol and linalool is available [27].
- The yields of the known terpene hydrocarbons are very low: myrcene (approx. 0.2 %), β -caryophyllene (under 1 %) and α -humulene (under 1 %).
- 2- and 3-methylbutyl-2-methylpropanoate do not provide a clear picture. An increase – under circumstances even variety-

specific – cannot be excluded.

- The average yields of isobutyl isobutyrate (+ 55 %) and 2-undecanone (+ 53 %) are similar, isoamyl propanoate yields higher with 83 %.

- 2 to 13 % of cubanol are transferred into beer

Terpene alcohols may transfer into each other. If this is limited to the pathways that were described for geraniol to citronellol and linalool, nerol respectively to linalool and further to terpineol in model solutions [28] or also may react the other way round wasn't investigated yet.

It is interesting to compare with transfer rates obtained when dosing into the hot wort. There are different informations for linalool. Late hopping brings transfer rates of up to 60 % [1, page 274, 18, 29]. Geraniol shows lower transfer rates [29].

When critically considering transfer rates, the difficulty lies in the fact that transformations in a medium with intact yeast enzymes can be manifold. Richly abundant methyl esters of the hops can become ethyl esters. Terpene alcohols like linalool, geraniol, nerol, citronellol and α -terpineol transform alternatingly into each other. Esters of these terpene alcohols can be split [26]. All these processes depend on many parameters like yeast strain and yeast cell count, temperature of the beer and contact time. After all, differences in the enzymatic potential of different yeasts (e.g. bottom-fermenting, ale and other top-fermenting yeasts) maybe the reason why the aroma of hop varieties is perceived differently. It would be useful to compare the effects of dry hopping in a beer with yeast, a similar filtrated beer without yeast and at least an enzymatically inactivated beer.

Sulfur compounds are found in hops with special aromas [1, page 275, 30]. The Cascade variety contains relevant quantities of 4-methyl-4-sulfanyl-2-pentanone (= 4-mercapto-4-methylpentan-2-one = 4MMP). In a study, a transfer rate of 64 % has been established with late hot hopping of Cascade [28]. The resulting concentration in the beer of 0.0017 $\mu\text{g/l}$ was significantly above the threshold value of 0.00055 $\mu\text{g/l}$. The analyses required for this were not available for these tests.

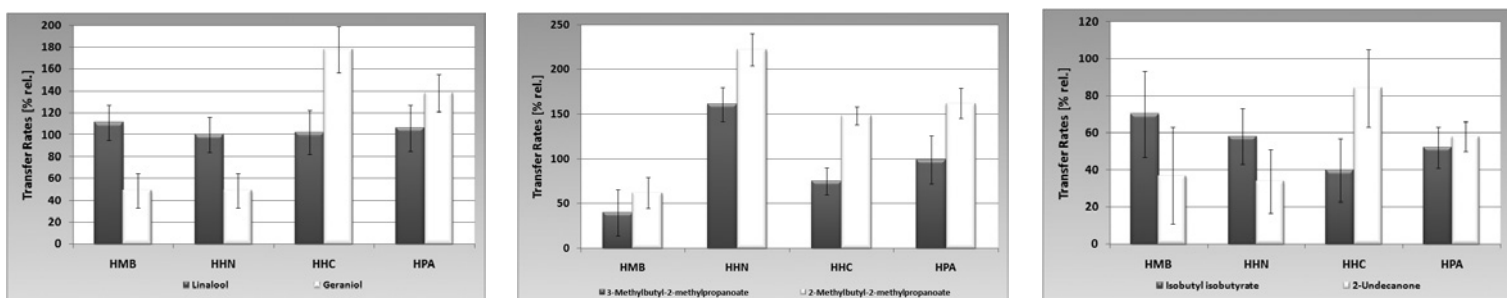
**Fig. 4–Fig. 6:** Transfer rates of 6 aroma components by dry hopping in % relative

Table 13 Hop aroma components in fresh and aged beers (100 days/20 °C); average of 5 beers in µg/l; changes in % rel.

Substance	fresh [µg/l]	aged [µg/l]	Change [% rel.]
Linalool	76,6	92,4	+21
Geraniol	36,0	69,0	+92
Nerol + Citronellol	40,0	8,6	-78
Myrcene	14,0	3,4	-76
β-Caryophyllene	1,7	0,9	-47
α-Humulene	5,3	2,9	-45
Ocimene	2,8	0,8	-71
Isoamyl propanoate	47,3	15,9	-66
3-Methylbutyl-2-methylpropanoate	42,8	17,7	-59
2-Methylbutyl-2-methylpropanoate	406,2	117,4	-71
Isobutyl isobutyrate	42,3	34,1	-19
2-Undecanon	15,7	4,4	-72
Caryophyllene alcohol	0,8	1,3	+63
Caryophyllenoxide	21,0	10,6	-50
Humulenepoxide I	8,7	4,3	-51
Humulenepoxide II	3,1	3,2	+3

6 Ageing of beers

The four dry-hopped beers and the reference beer were stored in the dark for 100 days at ambient temperature (20 °C ± 1 °C) and analysed for hop aroma components afterwards. An overview showed no significant differences in all 5 beers' deterioration, consequently an averaged evaluation of all 16 substances before and after ageing was done.

Table 13 shows the average contents of the 16 components in the 5 beers analysed in the fresh as well as the aged beer. Terpene alcohols linalool and especially geraniol increase during storage by a total of 46.8 µg/l. In contrast, nerol and citronellol decreased by 31.4 µg/l. The loss of terpenes myrcene, β-caryophyllene, α-humulene and ocimene can be explained by adsorption from the sealing compound of crown caps [10]. Chemical reactions, however, must also be taken into account as caryophyllene alcohol increases.

The average reduction of esters is considerably above 50 %. It can be assumed that even in filtrated beer there are vital yeast

Table 14 Average scores from five characteristics of beer from 1 (not or hardly noticeable) to 10 (most intensely noticeable) and personal preference from 1 (most preferred) to 5 (least preferred)

Criteria	Control	HMB	HHN	HHC	HPA
Intensity of hop aroma	4,3	6,5	6,3	7,2	6,2
Quality of hop aroma	6,0	6,7	6,5	6,0	6,3
Palatefulness	4,4	6,0	5,8	6,1	5,9
Quality of bitterness	6,0	6,5	6,5	6,0	6,4
Preference (average value)	3,6	2,3	2,4	3,8	3,0
Preference (ranking)	4	1	2	5	3

enzymes, as long as no deactivation e.g. by heat has occurred. Also, transesterification into ethyl ester is possible as well as non-enzymatically induced chemical reactions.

It is obvious that due to such intensive changes in analytical values sensorial impressions are subject to change, too. This applies to intensity as well as type and quality of hop aroma. In this sector, more intensive research work is essential.

7 Sensory results

Tasting dry hopped beers implicates difficulties. Dry hopped beers cannot be assessed simply using the same sensory methods developed for normal hopped beers. When evaluating a tasting scheme for dry hopped beers subjective preferences must be taken into account. It is not sufficient comparing only mean values, also the distribution of the data may play an important role.

The 5 beers were tasted by a panel of randomly selected consumers (30 members). The latter convened after the beers had been in cold storage (+5 °C) for three months and had to evaluate the following criteria:

- Intensity and quality of hop aroma from 1 to 10
- Body/palatefulness from 1 to 10
- Quality of bitterness from 1 to 10

In addition, they were asked to give their personal preferences from 1 (most preferred beer) to 5 (least preferred beer). Table 14 shows the average scores.

In all interpretations it must be taken into account that the reference beer was already richly hopped only with aroma hops (HHT and HHA). Figures 7 to 9 show the sensory results with 95 % confidence interval.

The following can be concluded:

- For intensity of hop aroma the reference beer is clearly behind the dry hopped beers. The highest value - admittedly within the confidence interval - was achieved by the beer with Hallertau Blanc. Dry hopping not by weight but by oil content (1.5 ml/hl) was useful here as proven by the relatively even intensity of the hop aroma. Dosing only by weight would have impaired the result.
- No significant differences were found for the quality of the hop aroma. The reference beer with its late hopping is also on the same level as the dry hopped beers. The on average greater confidence intervals appear to be logical: For the quality of the hop aroma, the individual senses of the testers play a greater role than the more quantitative characteristic of the intensity.
- Results for palatefulness, body: At first it might

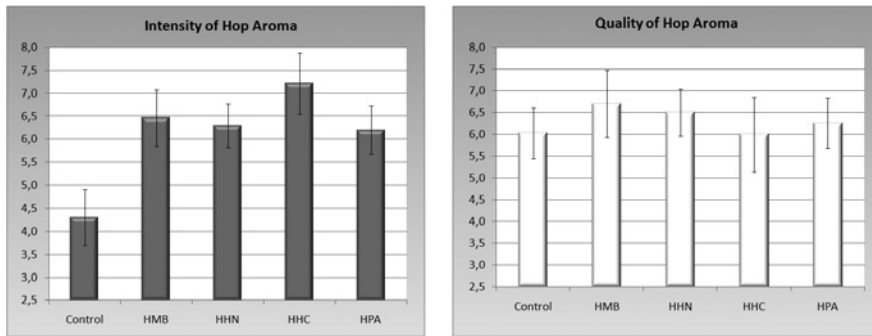


Fig. 7 Intensity (left) and quality (right) of the hop aroma of the five test beers with scores from 1 to 10

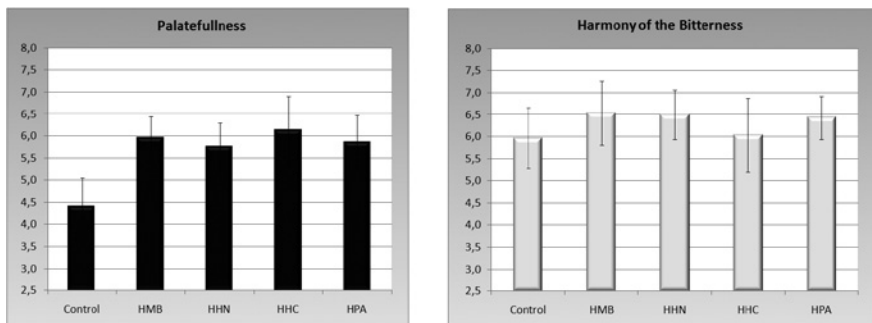


Fig. 8 Body/palatefulness (left) and quality of the bitterness (right) of the five test beers with scores from 1 to 10

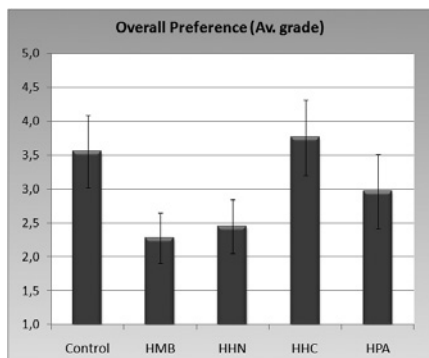


Fig. 9 Personal preference of the five test beers (from 1 to 5)

seem surprising that the reference beer did considerably less well (4.4 compared with the average of 5.9). By dry-hopping however, certain substances are dissolved as proven with the polyphenols. Indeed the observation seems plausible considering the interaction of these polar substances with non-polar substances like the dissolved α -acids or possibly even accompanying bitter substances [1, pages 212–217].

- The quality of bitterness is not negatively influenced by dry hopping with any of the varieties used, although the α -acid content increased. This is contradictory to other results [13].
- Regarding personal preference, the HMB and HHN beers differed only slightly and ranked significantly in front of the reference beer and the HHC beer. The Polaris beer lies in the middle.

The personal preference results were subjected to a Kramer’s rank sum test [31], which permits the following statistically significant statements:

- The HMB beer scores better than the reference, HHC and HPA beers.
- The HHN beer is in front of the reference and HHC beers.
- The reference beer lies behind the HMB, HHN and HPA beers.

The testers voluntarily noted their personal impressions whereby no criteria were specified. Table 15 shows the descriptions that were submitted more than once.

Comments like “fruity” are predominant. The interpreted synonyms of the designations of the hops (mandarin, melon, wine, fresh mint, glacier ice) were seldom used.

8 Summary

The transfer rates of some hop substances with dry hopping were determined by comparing a pale lager beer richly hopped in four stages with aroma hops with four additionally dry hopped beers. The four new breeds from Huell, *Mandarina Bavaria*, *Hüll Melon*, *Hallertauer Blanc* and *Polaris*, were dosed after main fermentation in the form of ground hop cones in the identical base beer in the storage tank. The quantities are the equivalent in each case to 1.5 ml hop oil per hectolitre. The result was 5 beers brewed according to the same recipe which differed only in the dry hopping.

Table 15 Descriptions of the 5 beers; figures in brackets = number of comments submitted

Control (6)	Mandarina Bavaria (12)	Hüll Melon (9)	Hallertauer Blanc (15)	Polaris (10)
4 x pleasant/hoppy	5 x fruity	2 x fruity	5 x fruity	3 x fruity
	3 x citrus	3 x sweet	2 x mango	2 x fresh, refreshing minty note
	2 x mandarin	2 x melon	2 x wine	1 x wine
	1 x melon	1 x mandarin	1 x melon	

From the beer analyses conducted the following conclusions can be drawn:

- Of the α -acids, 4 to 5 % relative are proven to be in the dry hopped beer.
- The transfer rate of total polyphenols is 50–60 % and about 70 % for low-molecular polyphenols.
- Single polyphenols behave differently; procyanidin B3, catechin, caffeoylquinic acid and two glucosides transfer at 100 %, and the transfer rate of two malonylhexosides is 60–70 %. Whether, for example, enzymatic transformations of the glucosides take place, has to be checked.
- The aroma substances analysed varied greatly. Minimal transfer rates (under 1 %) were registered for myrcene, β -caryophyllene and humulene. About 50% of Isobutyl isobutyrate and 2-undecanone are transferred. Two other esters analysed showed transfer rates of partly over 100 %. Ocimene and cubenol transfer at approx. 10 %.
- Linalool and geraniol are interesting to observe. Linalool showed steady transfer rates of 100 %, geraniol was at approx. 50 % in two beers and well over 100 % in the other two.

Extensive analyses are still necessary also for the aroma substances, in particular with regard to enzymatic reactions. However, initial reference points are determined how transfer rates differ and which substances transfer variety dependent into beer. In this way indications are given as to the form in which the noticeably individual compositions of the new varieties with special aromas (“flavour hops”) can be recovered in beer.

Hop aroma components change considerably during beer ageing and effect intensity as well as quality of hop aroma.

The tasting by a panel of 30 untrained consumers produced the following results:

- The intensity of the hop aroma is much more distinctive in the dry hopped beers. Dosing according to oil content results in a comparable intensity in the dry hopped beers.
- There was no significant preference regarding the quality of the hop aroma in any of the beers including the non-dry hopped reference beer. The personal preferences of the tasters are dominant.
- The palatefulness/body of the dry hopped beers was assessed to be significantly better than that of the reference beer, which might well be due to the dosing of polyphenols.
- There was a significant preference for the beers hopped with *Mandarina Bavaria* and *Hüll Melon*. The reference beer and the Hallertau Blanc beer lay significantly behind and Polaris was in the middle.
- The tasters could not detect any increase in the bitterness by dry hopping in these tests.

Acknowledgment

Filip Van Opstaele (KAHO St. Lieven/Gent) for the analysis of the aroma components in the beers.

Roland Schmidt and the NATECO₂ for the analysis of polyphenols in hops and beers and aroma components in hops.

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Received 03 July 2013, accepted 7 August 2013