Varietal Difference of Hop-Derived Flavour Compounds in Dry-Hopped Beers

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FLAVOUR COMPOSITION | Eleven selected flavour compounds in late hopped and dry hopped beers brewed with single hop varieties have been investigated. On the basis of the results, a comparison of varietal differences of hop-derived flavour compounds among eighteen hop varieties, correlations between certain monoterpene alcohols and their derivatives and the effect of hopping procedure on flavour compositions in beers were discussed. The following article summarises the research results which have already been published in BrewingScience, 2016, issue 1: January/February.

HOPS CONTAIN various flavour compounds, for example terpene hydrocarbons, terpene alcohols, esters, ketones, short-chain fatty acids, sulphur compounds, and so on [1, 2]. In the wort boiling process, these compounds are exposed to high evaporation and strong oxidative conditions. Flavour compounds are also affected by yeast fermentation, for example adsorption to foam and/or yeast cells, reduction, hydrolysis, esterification, biotransformation, and so on. Hop-derived flavour compounds in finished beer are present or are formed through complex brewing processes [3 - 6].

In recent years, craft brewers have been brewing unique beers, made with characteristic hops, all around the world. These hops can impart citrus and/or exotic fruit-like flavours to finished beer. A group of such characteristic hops is often referred to as "flavour hops" [7]. A beer brewed with such flavour hops has a very unique "varietal aroma." Several researchers have tried to identify variety-specific flavour compounds contributing to the varietal aroma derived from certain hops [3 - 4, 6, 8 - 20]. However, possible contributors to the varietal aroma of each flavour hops have not yet been fully investigated.

Among many flavour compounds derived from hops, the focus was on monoterpene alcohols (linalool, β-citronellol, nerol and geraniol) and their derivatives (geranyl acetate and linalool oxide), β-ionone and several esters (isobutyl isobutyrate, isoamyl isobutyrate, 2-methylbutyl isobutyrate and ethyl heptanoate) (fig. 1).

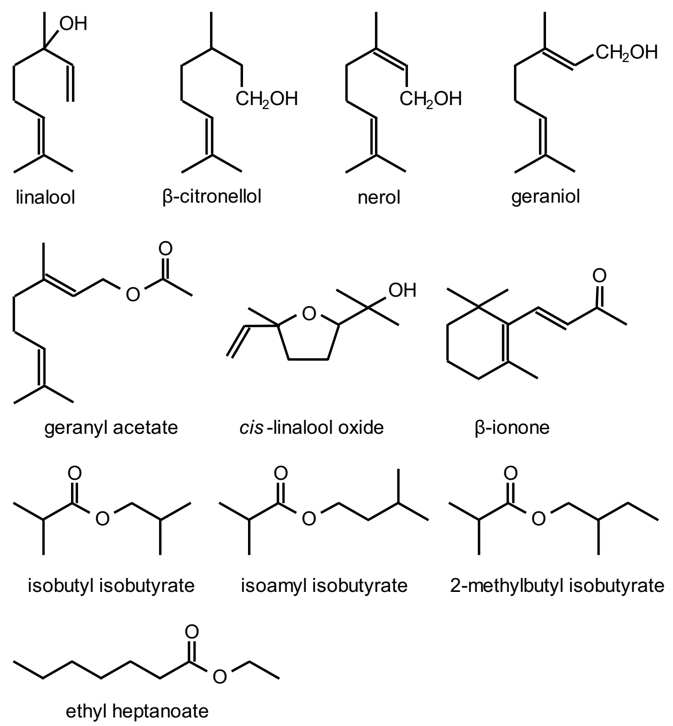

Fig. 1 Various flavour compounds derived from hops

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COMPOSITIONS OF HOP-DERIVED FLAVOUR COMPOUNDS IN TEST BEERS BREWED WITH U.S. AND GERMAN HOPS

hop variety		Apollo	Bravo	Citra	Mosaic	Summit	Hallertau Blanc	Hüll Melon	Mandarina Bavaria	Polaris
crop		2010	2010	2008	2012	2012	2012	2012	2012	2012
flavouring hop (g/L of wort)		0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
linalool	(µg/L)	27.8	34.0	52.9	50.9	60.2	51.3	28.1	42.1	62.6
β-citronellol	(µg/L)	11.8	31.9	17.3	24.3	34.5	32.1	15.0	17.8	43.8
nerol	(µg/L)	1.2	2.8	2.4	5.1	4.0	2.6	2.9	3.1	5.4
geraniol	(µg/L)	4.4	11.0	5.8	13.8	23.6	21.0	8.7	9.6	35.5
geranyl acetate	(µg/L)	2.2	2.9	1.6	2.0	3.7	2.5	1.0	1.4	5.8
cis-linalool oxide	(µg/L)	2.5	2.4	6.2	1.3	5.9	2.0	5.0	1.7	1.4
β-ionone	(µg/L)	0.1	0.1	0.1	tr	tr	0.1	0.1	0.1	tr
isobutyl isobutyrate	(µg/L)	1.4	1.3	0.9	10.1	2.8	10.0	25.2	22.4	54.0
isoamyl isobutyrate	(µg/L)	1.5	1.9	0.4	10.8	3.1	5.9	7.3	3.4	16.5
2-methylbutyl isobutyrate	(µg/L)	9.6	10.3	1.6	26.0	7.3	18.9	41.4	27.6	63.6
ethyl heptanoate	(µg/L)	2.5	2.8	2.6	5.7	2.6	2.7	3.1	4.4	8.8

Table 1

Possible contributors to hop varietal differences

Monoterpene alcohols, derived from hops have been identified by many researchers [3, 4, 8-14, 21-23]. Of all monoterpene alcohols, linalool is well known as a useful indicator of a hop-accented beer flavour. β-citronellol and geraniol are also important contributors to the lime-like flavour of hopped beer. In our previous studies [3, 4, 6], β-citronellol was almost absent in hops and wort and increased slightly during fermentation as a result of biotransformation from geraniol to β-citronellol brought about by brewing yeast. Though, in general, geraniol drops significantly during the first 3-4 days of fermentation, the concentration of geraniol and β-citronellol in beer could be enhanced as a function of the initial geraniol content in the wort; for example, by using hops rich in geraniol. In addition, an additive effect between linalool, geraniol and β-citronellol was noted. In this case, linalool was found to be a flavour enhancer, and flavour intensities of geraniol and β-citronellol could be raised when linalool was also present [3]. The flavour impression became lime-like when all three monoterpene alcohols co-existed [4].

Geranyl acetate contained in hops and beers has been mentioned in the literature [9, 13, 15, 21]. *Lam* et al. suggested that geranyl acetate could be hydrolysed to geraniol during fermentation [9]. In recent years, *Forster* et al. reported that beers dry-hopped with hops rich in geranyl acetate (Cascade, Hallertau Blanc and Polaris) contained small amounts of this compound and large amounts of geraniol [15]. This result strongly supports Lam's hypothesis.

It was assumed that linalool oxide would arise from oxidation of linalool during wort boiling [21, 24]. *Haley* et al. compared various flavour compounds in late-hopped and dry-hopped beer and reported that both trans- and cis-linalool oxide were found in late-hopped beer but not in dry-hopped beer [24].

β-ionone which has a violet-like floral flavour has been found in hops and beers [10, 21, 23, 25]. Haley et al. reported that only traces of β-ionone were found in late-hopped and dry-hopped beers using Styrian Golding hop extract [24]. On the other hand, *Kishimoto* et al. detected β-ionone in late-hopped beers brewed with Saazer, Hersbrucker and Cascade hops [10] and in dry-hopped beers brewed with a bitter variety [23].

Isobutyric esters including isobutyl isobutyrate, isoamyl isobutyrate and 2-methylbutyl isobutyrate were extensively found in various hops [16-17, 24,

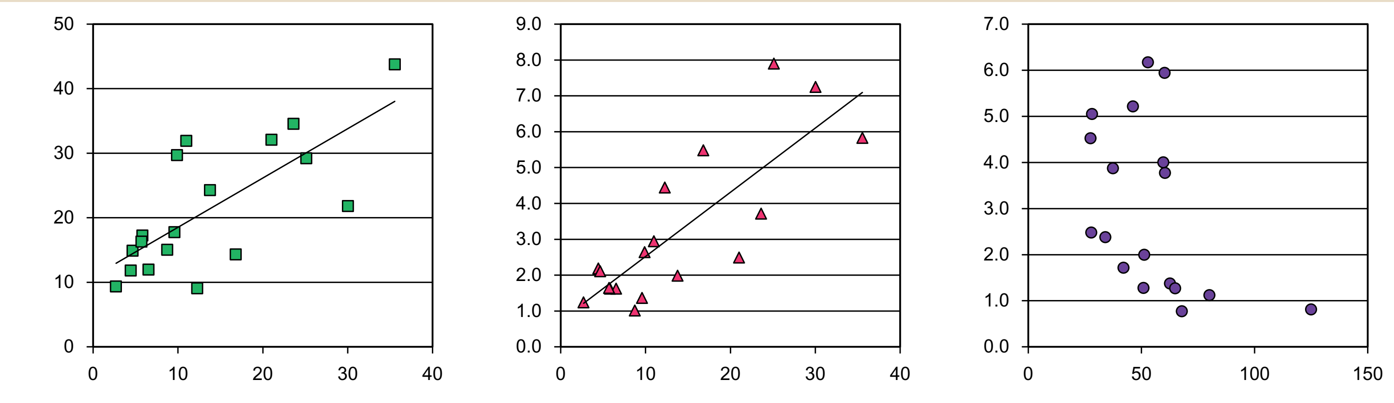

Fig. 2 Correlation between monoterpene alcohols and their derivatives

COMPOSITIONS OF HOP-DERIVED FLAVOUR COMPOUNDS IN TEST BEERS BREWED WITH NEW ZEALAND HOPS

hop variety		Kohatu	Motueka	Nelson Sauvin	Pacific Jade	Rakau	Riwaka	Southern Cross	Wai-iti	Wimea
crop		2012	2010	2012	2010	2012	2010	2010	2012	2012
flavouring hop (g/L of wort)		0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
linalool	(µg/L)	37.4	80.0	27.5	64.9	60.4	67.8	125.0	46.3	59.7
β-citronellol	(µg/L)	9.4	29.2	12.0	9.1	14.9	21.8	14.3	16.3	29.7
nerol	(µg/L)	1.6	2.0	2.6	1.1	2.1	1.5	1.2	2.2	2.4
geraniol	(µg/L)	2.7	25.1	6.5	12.3	4.7	30.0	16.8	5.7	9.9
geranyl acetate	(µg/L)	1.2	7.9	1.6	4.4	2.1	7.2	5.5	1.6	2.6
cis-linalool oxide	(µg/L)	3.9	1.1	4.5	1.3	3.8	0.8	0.8	5.2	4.0
β-ionone	(µg/L)	0.1	0.1	0.1	0.1	tr	0.1	tr	0.1	tr
isobutyl isobutyrate	(µg/L)	2.7	4.4	9.3	18.5	17.4	9.3	17.9	6.1	21.1
isoamyl isobutyrate	(µg/L)	0.6	1.2	1.6	3.1	1.8	4.0	5.3	1.4	1.7
2-methylbutyl isobutyrate	(µg/L)	3.3	18.1	17.8	78.1	26.3	38.6	103.6	16.4	35.2
ethyl heptanoate	(µg/L)	2.4	1.8	3.0	3.9	3.7	1.6	3.5	3.7	3.5

Table 2

26-28]. These esters have a green apple, apricot-like flavour [16-17]. Traditional bitter hops and modern high-alpha hops, for example Northern Brewer, Nugget and Magnum, contain relatively high amounts of these compounds. Traditional aroma hops, German aroma hops, for example Hallertauer Tradition, contained these compounds while Saaz and Lublin had low levels [16-17, 28]. *Seaton* et al. reported that isobutyric esters could be unstable during boiling and fermentation [29]. In fact, commercial beers brewed by means of kettle hopping contain only small amounts of these compounds [16]. On the other hand, these compounds are found in late-hopped and dry-hopped beer [29-31]. Isobutyric esters are thought to contribute to some of the special flavours of dry-hopped beers.

Ethyl heptanoate can be formed by methyl heptanoate derived from hops [24]. Several researchers reported finding methyl heptanoate in raw hops [26-27]. Heptanoic acid is a minor metabolite formed during yeast fermentation. Therefore, methyl heptanoate in hops might behave as a precursor of ethyl heptanoate.

In this study, monoterpene alcohols (linalool, β-citronellol, nerol and geraniol) and their derivatives (geranyl acetate and linalool oxide), β-ionone and various esters (isobutyl isobutyrate, isoamyl isobutyrate, 2-methylbutyl isobutyrate and ethyl heptanoate) were analysed and varietal differences of hop-derived flavour compounds in beers made with U.S., German and New Zealand hops were compared.

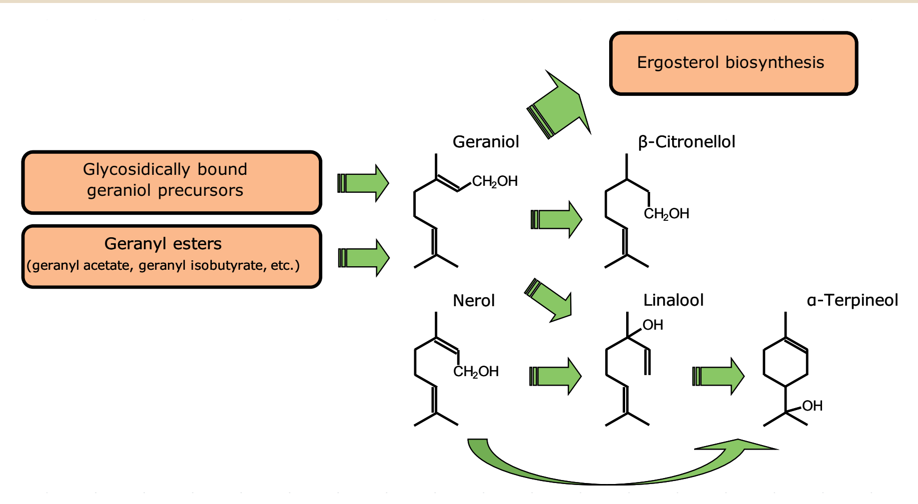

Fig. 3 Biotransformation pathway of monoterpene alcohols induced by brewing yeast (pursuant to [3, 4, 6, 9, 13, 15, 38-40])

Varietal differences of hop-derived flavour compounds

Test beers were brewed using late hopping and single hop varieties. The U.S. hop varieties (Apollo, Bravo, Citra [32, 33], Mosaic [34] and Summit), the German varieties (Hallertau Blanc, Hüll Melon, Mandarina Bavaria and Polaris) [35] and the New Zealand varieties (Kohatu, Motueka, Nelson Sauvin [36, 37], Pacific Jade, Rakau, Riwaka, Southern Cross, Wai-iti and Wimea) were used. The compositions of hop-derived flavour compounds obtained by SPME-GC-MS analysis of these beers are shown in tables 1 and 2. Major differences in compositions were analysed depending on hop variety.

When comparing these eighteen hop varieties, major variations in the amounts of monoterpene alcohols (linalool, β-citronellol and geraniol), their derivatives (geranyl acetate and cis-linalool oxide) and esters were found. As a result, it was assumed that these compounds were useful for distinction between "flavour hop" varieties. Of all monoterpene alcohols, amounts of nerol were smaller than those of other monoterpene alcohols and fluctuated within a small range (1.1 - 5.4 µg/L). Only traces of β-ionone were measured (max. 0.1 µg/L) in all test beers. In addition, it was reported that the thresholds of nerol and β-ionone were 80 µg/L [3] and 0.6 µg/L [23]. It was thus assumed that the contribution of nerol and β-ionone to the hop varietal difference was smaller than those of other compounds.

SPME-GC-MS is a simple and useful method for analysing various flavour compounds. A certain category of compounds, for example volatile thiols, could not be analysed with this method. It has been reported that volatile thiols are important contributors to the varietal aroma of several flavour hop varieties [11-12, 16-20]. It might thus be possible to assemble improved varietal compositions of hop-derived flavour compounds using a combination of several analytical methods.

Monoterpene alcohols and their derivatives

Figure 2 shows the scatter plot graphs using the data from tables 1 and 2. The correlation between geraniol, β-citronellol and geranyl acetate was relatively close. The correlation coefficient between geraniol and β-citronellol was 0.7325 and that between geraniol and geranyl acetate was 0.8108.

Figure 3 shows the biotransformation pathway of monoterpene alcohols brought about by brewing yeast based on several previous publications [3-4, 6, 9, 13, 15, 38-40]. β-citronellol was almost absent in hops and wort and gradually increased during fermentation resulting from biotransformation from geraniol to β-citronellol induced by yeast [3-4, 6]. Though free geraniol in initial wort drops substantially during the first 3 - 4 days of fermentation [3-4, 6, 9, 38-40], free geraniol was supplied by glycosidically bound geraniol precursors [3, 6] and/or geranyl esters [9, 13, 15].

The results shown in figure 2 support the biotransformation pathway shown in figure 3. Previous studies indicated that geraniol contents in finished beer depended not only on the initial level of geraniol in wort but also on release of geraniol by precursors (glucosidically bound precursor [3, 6] and/or geranyl esters [9, 13, 15]). Parts of geraniol released were transformed to β-citronellol during secondary fermentation [3, 6].

On the other hand, the correlation between linalool and cis-linalool oxide was relatively low (fig. 4). Haley et al. suggested that linalool oxide might be formed by linalool under oxidative conditions during wort boiling and that linalool oxide was detected in late-hopped beer though not in dry-hopped beer [24]. However, the result in figure 4 indicates that the amount of linalool oxide in beer might not depend on the amount of linalool derived from hops. It was assumed that linalool oxide might be contained in raw hops.

Effect of hopping procedure on flavour profiles of beers

In addition, test beers were brewed using dry-hopping on day 3 (dry 1) and day 6 (dry 2) of primary fermentation using single hop varieties, Hüll Melon, Mandarina Bavaria and Mosaic. Flavour profiles between late-hopped and two dry-hopped beers were compared (table 3).

Of all monoterpene alcohols, the level of geraniol in finished beer rose when delaying the timing of hop addition (dry 1 and dry 2), resulting from the fact that geraniol was reduced significantly during the yeast growth phase [3-4, 6, 9, 38-40]. In our previous study, it was possible to raise geraniol levels in finished beer only when delaying the timing of hop addition (not in dry hopping) so as to bypass the yeast growth phase [6]. Changes in levels of other monoterpene alcohols and geranyl acetate were not more significant than that in geraniol. Interestingly, cis-linalool oxide increased when using dry hopping. This finding would support the assumption that cis-linalool oxide is contained in raw hops. Only traces of β-ionone were found (max. 0.2 µg/L) under all hopping-conditions. Amounts of isobutyric esters and ethyl heptanoate rose when delaying the timing of hop addition.

Conclusions

It was assumed that monoterpene alcohols (linalool, β-citronellol, nerol and geraniol) and their derivatives (geranyl acetate and cis-linalool oxide), β-ionone and several esters (isobutyl isobutyrate, isoamyl isobutyrate, 2-methylbutyl isobutyrate and ethyl heptanoate) are potential contributors to hop varietal aroma. Compositions of these compounds in test beers brewed using eighteen hop varieties have been analysed and compared.

It was found that concentrations of linalool, β-citronellol, geraniol, geranyl acetate, cis-linalool oxide, isobutyl isobutyrate, isoamyl isobutyrate, 2-methylbutyl isobutyrate and ethyl heptanoate varied extensively in finished beers brewed with eighteen hop varieties. However, nerol and β-ionone might not almost contribute to the hop varietal difference.

New characteristic hop varieties are currently bred all over the world. Such hops and beers brewed with these hops are analysed using various means. It could be assumed that coming up with a useful parameter for distinguishing hop-derived flavour compounds in beer would be expedient for identifying differences between hop varieties and would make a real contribution to hop-derived varietal aroma in beer. It might thus be possible to assemble improved varietal compositions of hop-derived flavour compounds to identify contributors using a combination of several analytical methods.

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COMPOSITIONS OF HOP-DERIVED FLAVOUR COMPOUNDS IN LATE-HOPPED AND DRY-HOPPED BEERS BREWED WITH HÜLL MELON, MANDARINA BAVARIA AND MOSAIC

hop variety		Hüll Melon			Mandarina Bavaria			Mosaic		
crop		2012			2012			2012		
flavouring hop (g/L of wort)		0,8			0,8			0,8		
hopping prodedure		late	dry 1[a]	dry 2[a]	late[a]	dry 1[a]	dry 2[a]	late	dry 1[a]	dry 2[a]
linalool	(µg/L)	28.1	16.9	17.0	42.1	26.1	28.2	50.9	34.6	43.1
β-citronellol	(µg/L)	15.0	19.1	11.7	17.8	15.6	15.1	24.3	19.5	20.6
nerol	(µg/L)	2.9	4.4	4.4	3.1	4.3	6.1	5.1	5.1	6.4
geraniol	(µg/L)	8.7	12.9	15.8	9.6	13.2	16.9	13.8	17.1	21.2
geranyl acetate	(µg/L)	1.0	2.0	1.2	1.4	2.0	2.0	2.0	2.5	3.6
cis-linalool oxide	(µg/L)	5.0	11.2	10.4	1.7	5.8	6.5	1.3	4.7	5.3
β-ionone	(µg/L)	0.1	0.2	0.1	0.1	0.2	0.2	tr	0.1	0.2
isobutyl isobutyrate	(µg/L)	25.2	26.0	39.5	22.4	19.7	27.8	10.1	9.3	13.6
isoamyl isobutyrate	(µg/L)	7.3	8.5	12.2	3.4	3.3	4.6	10.8	11.8	16.3
2-methylbutyl isobutyrate	(µg/L)	41.4	42.0	57.0	27.6	24.1	31.6	26.0	22.9	30.9
ethyl heptanoate	(µg/L)	3.1	3.3	3.3	4.4	4.7	6.4	5.7	5.4	7.6

Table 3 *a) dry-hopped on day 3 (dry 1) and day 6 (dry 2) of primary fermentation*

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