Key-role of humulinones, polyfunctional thiols and terpenols in Belgian dry-hopped beers

Carlos Silva Ferreira, Eloi Thibault de Chanvalon and Sonia Collin

Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute ELIM, Faculté des Bioingénieurs, Université catholique de Louvain, Croix du Sud, 2 box L7.05.07, B-1348 Louvain-la-Neuve, Belgium

Introduction

The addition of hop during the late stages of the brewing process brings singular characteristics to the beer. Polyphenols and oxidation products of hop humulones like humulinones and hulupones can modify their bitterness and astringency. Terpenols, polyfunctional thiols and bound adducts (glucosides, cystein- and glutathionyl-thiol adducts) can impart new flavors to such beers. However, hop characteristics alone may not entirely predict the sensorial profile of dry-hopped beers. The aim of this work was to better describe bitterness and flavors of 21 Belgian dry-hopped beers.

Methods

Twenty one Belgian dry-hopped beers (DHB) were investigated in this work: Orval (A), Bastogne Pale Ale (B), La Trouffette Blonde (C), Vedett Extra Ordinary IPA (D), IV Saison (E), V Cense (F), Leffe Royale Cascade IPA (G), Leffe Royale Mapuche (H), Leffe Royale Mount Hood (I), Houblon Chouffe (J), Taras Boulba (K), St. Feuillien Saison (L), St. Feuillien Grand Cru (M), Guldenberg (N), Houppe (O), Duvel Triple Hop 1 – Amarillo (P), Duvel Triple Hop 2 – Citra (Q), Duvel Triple Hop 3 – Sorachi Ace (R), Duvel Triple Hop 4 - Mosaic (S) Duvel Triple Hop 5 - Equinox (T), and Duvel Triple Hop 6 – HBC291 (U), and compared to five hoppy non-dry-hopped beers (NDHB): Saison Silly (V), Saison Voisin (X), Saison 1900 (Y), Biolegère (W) and XX Bitter (Z). Bitter compounds were isolated from samples by solid phase extraction (SPE) and analyzed by RP-HPLC-UV (gradient from 6 to 95% of methanol – λ = 270 nm for isohumulones and humulinones, 320 nm for hulupones and 314 nm for humulones). Hop Aroma compounds were isolated from DHB by Solvent Assisted Flavor Evaporation (SAFE)[6], extracted with CH_2Cl_2 and analyzed by GC-MS. Polyfunctional thiols were isolated from beers by pHMB extraction[2,7] and analyzed by GC-PFPD.

Standard analyses

Table 1. Standard analyses of DHB.

Beer	Alcohol content (% ABV)	Original extract (ºP)	Apparent attenuation (ºP)	pH	Color (ºEBC)	Total polyphenols (mg/L)	Total flavanoids (mg/L)	Degree of polymerization	Bitterness (BU)
A	6,6	16,5	97,7	4,29	15,5	81,2	44,1	1,8	25,2
B	5,1	13,2	77,6	4,35	13,5	170,2	52,3	3,3	42,2
C	6,1	15,1	81,2	4,42	11,5	244,8	52,6	4,7	34,6
D	5,4	13,5	81,4	4,4	14,5	171,4	47,7	3,6	36,1
E	5,4	12,4	90,0	4,45	12,5	166,5	35,6	4,7	40,3
F	6,0	13,4	89,8	4,54	31,5	155,4	27,0	5,8	30,4
G	7,2	15,5	93,7	4,42	10,0	119,5	25,5	4,7	31,8
H	7,2	15,6	93,0	4,36	16,5	132,0	27,6	4,8	28,8
I	6,8	17,6	78,3	4,37	43,5	137,1	28,2	4,9	28,5
J	8,3	18,4	90,1	4,32	11,0	341,3	71,8	4,8	44,1
K	4,4	10,5	85,3	4,45	5,0	333,1	66,7	5,0	57,9
L	6,6	15,6	85,2	4,46	14,5	300,9	54,4	5,5	25,7
M	9,1	20,6	88,7	4,5	8,3	255,6	53,0	4,8	29,8
N	7,4	18,8	79,7	4,44	14,5	360,6	61,1	5,9	33,8
O	7,4	17,8	84,1	4,03	29,0	287,6	58,3	4,9	42,3
P	9,1	19,6	93,4	4,05	5,5	221,4	65,3	3,4	33,1
Q	9,3	19,7	94,2	4,04	6,0	239,4	69,3	3,5	35,2
R	9,3	19,6	94,2	4,01	6,0	235,3	69,8	3,4	32,0
S	9,1	19,2	94,8	4,03	6,2	250,9	72,7	3,5	37,0
T	9,3	19,8	93,6	4,05	6,2	292,5	73,7	4,0	40,9
U	9,4	19,7	95,2	4,13	5,7	225,1	73,9	3,0	32,0

Bitterness

Humulones were found in all DHB, with concentrations ranging from 1.1 (A) to 7.1 (L) mg/L (Figures 1 and 2). On the opposite, NDHB contained less than 3 mg/L. Humulinones ranged from 1.0 (A) to 13.1 (D) mg/L in DHB, while values under 2.1 mg/L were found for NDHB. No correlation between humulones and humulinones concentration was observed (R^2 = 0,35). Hulupones were detected only as traces in all beers, with no significant difference between DHB and NDHB samples.

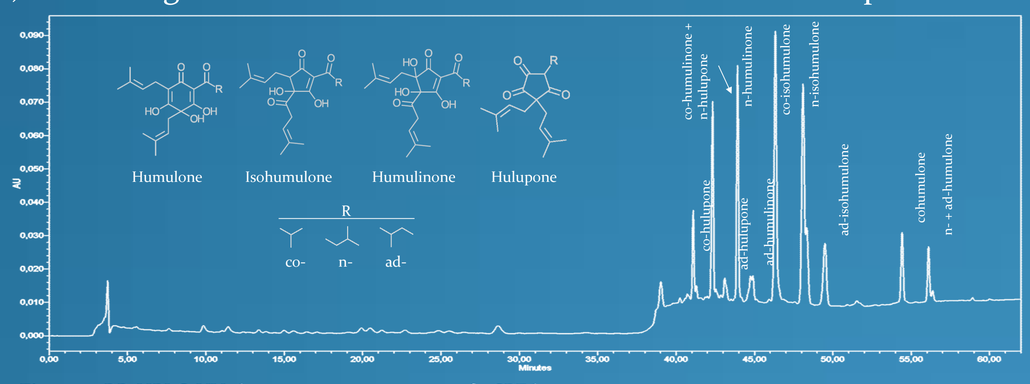

Figure 1. RP-HPLC-UV chromatogram at 270 nm of a SPE beer extract.

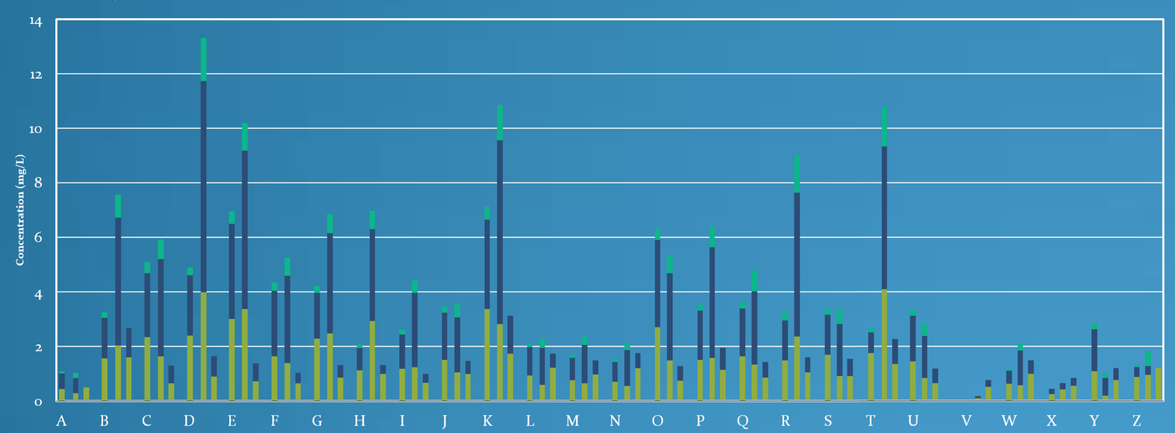

Figure 2. Humulones, humulinones and hulupones concentrations in DHB (A – U) and NDHB (V – Z). Bars from left to right : humulones, humulinones, and hulupones. For each bar: co-, n- and ad- analogs.

Humulinones stability was investigated throughout 90 minutes of boiling in model medium (pH = 5,6) and 12ºP wort (Figures 3 and 4). Their degradation at high temperatures explains the low amounts found in NDHB.

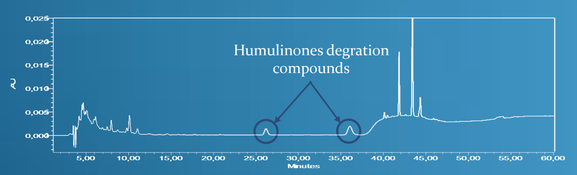

Figure 3. RP-HPLC-UV chromatogram at 270 nm of model medium at 90 minutes of boiling.

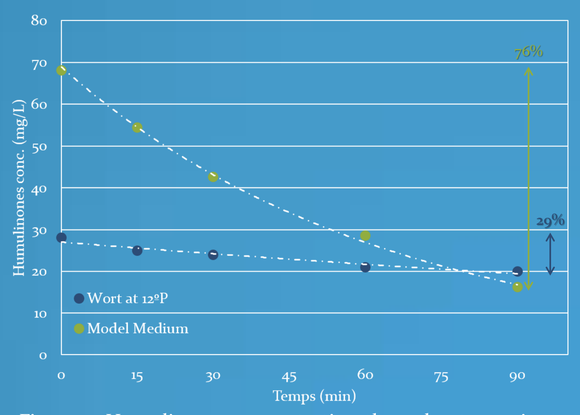

Figure 4. Humulinones concentration throughout 90 minutes of boiling in model medium (spiking of standards) and 12ºP wort (oxidized Saaz addition).

Aroma compounds

DHB samples presented a nice diversity of hop derived compounds. D, S and T showed the highest number of hop-derived compounds (table 2). Linalool concentrations were above its threshold in most samples. Beer Q, dry-hopped with Amarillo, have the highest amount, 493.6 µg/L. Geraniol was often found in concentrations close to its threshold.

Table 2. Hop flavors in DHB analyzed by SAFE – GC/MS (concentrations in µg/L of beer).
Present. Absent.

	Compound	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	Odor
Esters	2-Methylbutyl isobutanoate																						fruity
	Ethyl heptanoate				6.0								5.3		5.8	6.6	19.2	16.8		6.2	19.4	14.1	fruity
	Isobutyl isobutanoate				63.3		26.1								19.2	20.4		28.4			80.1		pineapple
	Ethyl isovalerate	63.4			21.2				16.9							10.0		22.9		19.7	114.2		apple, candy
Terpenoids	Linalool	65.4			424.8		223.3		99.9	323.0		114.9	86.0		129.4	280.0	249.7	493.6	135.8	188.4	498.5	425.5	flowery, coriander
	Geraniol				41.3		13.3		16.5	20.3		6.8				18.6	28.2	24.4	7.1	26.9	52.6	15.0	flowery
	α-Terpineol	18.8			61.6		24.4		14.6	50.6		23.9	39.0		30.0	35.0	51.6	75.6	32.9	46.1	113.5	65.8	moist orange
	Methyl geranate				72.5		15.6									41.7	6.4	30.9		17.3	19.3	8.5	fruity. floral
	Citronellol																						citrus, flowery
	Myrcene															9.2					16.6		herbs, resinous
Secondary alcohol	2-Undecanol																						floral, citrus
Acids	Isovaleric acid																						sweat, rancid
	2-Methylbutanoic acid																						sweaty, rancid

Figure 5 shows how different can be the polyfunctional thiol profile of a beer when different dual hops are used in dry hopping. S was the richest beer while R and U presented none of the selected compounds. In addition, the unexpected high amounts of 3S4MPol found in Q, and S come probably from the liberation from precursors (cystein- and glutathionyl- thiol adducts)[9].

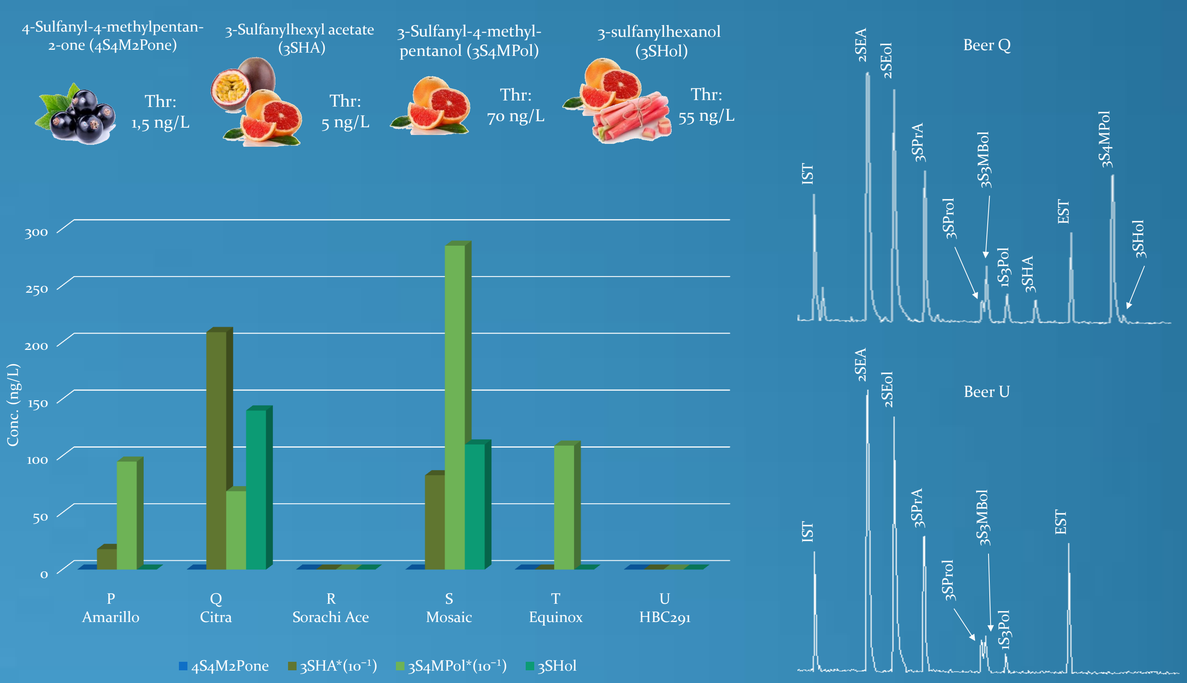

Figure 5. Left: amounts of polyfunctional thiols determined by GC-PFPD in beers (µg/L, IST equivalents for compounds carrying the * superscript). True 3SHA and 3S4MPol concentrations are 10 times higher than depicted. Right: GC-PFPD chromatogram of pHMB extracts issued from beers Q and U.

Conclusion

Rarely found in NDHB, humulinones are confirmed to be key bitterness compounds of DHB. Some specific aroma compounds (thiols, esters, terpenoids, acids and alcohols) allow us to identify which hop variety was used for dry hopping.

References

[1] Collin S, Nizet S and Gros J. Cerevisia, 2012, 36, 119-124.
[2] Gros J, Nizet S and Collin S. J. Agric. Food Chem., 2011, 59, 8853-8865.
[3] Gros J, Thi Thu H T and Collin S. J. Inst. Brew., 2013, 119, 221-227.
[4] Thi Thu H T, Kankolongo Cibaka M-L and Collin S. J. Am. Soc. Brew. Chem., 2015, 73, 61-70.
[5] Algazzali and Shellhammer, J. Am. Soc. Brew. Chem., 2016, 74, 36-43.
[6]Engel W, Bahr W and Schieberle P. Food Res. Technol., 1999, 209, 237-241.
[7] Tominaga Murat M-L and Dubourdieu D. J. Agric. Food Chem. 1998, 46, 1044-1048.
[8] Kankolongo Cibaka M-l, Gros J, Nizet S and Collin S. J. Agric. Food Chem., 2015, 63, 3022-3030.
[9] Kankolongo Cibaka M-L, Decourrière l, Lorenzo-Alonso C J, Bodart E, Robiette R and Collin S. J. Agric. Food Chem., 2016, 64, 8572-8582.

Acknowledgements

We are indebted to Brasserie d'Orval, Brasserie de Bastogne, Duvel Moortgart Brewery, Brasserie de Jandrain-Jandrenouille, AB-InBev Belgium, Brasserie d'Achouffe, Brasserie de la Senne, Brasserie St-Feuillien, Brasserie de Ranke and Brasserie artisanale de Namur, which kindly provided us the beer samples, and to Martin Biendl from Hopsteiner for the analytical standards of humulinones and hulupones.