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Reproducibility Trials in a Research Brewery and Effects on the Evaluation of Hop Substances in Beer

Part 1: Reproducibility in fresh beers

The brewing industry, suppliers and universities have pilot breweries with different sizes and varying requirements to study technological or raw material related questions. Thereby the reproducibility of the brewing system as well as of the analytical evaluation of the samples drawn have to be payed closer attention to. This study describes the methodology and results of a reproducibility investigation on a 2hl pilot plant with particular focus on hop substances in the beers produced.

Descriptors: reproducibility of brewing trials, hop aroma substances, bitter substances, fermentation by-products, polyphenols, ageing aldehydes

1 Introduction

Trial brews, even on a small scale, are comparatively time consuming and expensive. Therefore rarely multiple brews are made in a test series and only chemical analyses are carried out as two-fold or three-fold determinations.

In our case, numerous test series have already been made in the research of hopping parameters. Usually a series included multiple brews which differed only in the hopping. General beer analyses are well suited for checking whether production of the wort with subsequent fermentation, storage and bottling are reproducible or whether deviations are discernible. Years of working with the St. Johann Research Brewery has yielded enough data on the reproducibility of the plant. Since its commissioning in 1998 several publications are testimony to the general suitability of the plant [1–4]. Examples of mean values and standard deviations of general beer analyses are given in table 1. A special series of brews for determining the reproducibility is nevertheless lacking to date, a situation that is to be put right by this presentation of trial brews. Here, the focus is trained in particular on hop substances. Sensory analyses are not included in this study.

Table 1 Mean values and standard deviations (SD) of general beer analyses in two different brewing programs

		Series 1 (n=7) [2]		Series 2 (n=7) [2]		Series 3 (n=5) [3]	
		MEAN	SD	MEAN	SD	MEAN	SD
OG	%mas	7.98	0.20	7.95	0.09	11.65	0.16
ABV	%vol	1.15	0.05	1.13	0.05	5.21	0.13
pH		4.44	0.04	4.47	0.04	4.49	0.05
Bitterness	IBU	15.9	0.4	19.5	0.6	27.4	1.1

The extensive analytical data obtained from these trials will be discussed in three parts:

- Part 1: Reproducibility in fresh beers
- Part 2: Reproducibility in beers aged differently
- Part 3: Technological results and conclusions

2 Materials and methods

2.1 Brewery setup

The fully automated 2 hl pilot brewery that was primarily built as a two pieces brewhouse was expanded in 2012 and is state of the art thanks to a Siemens PCS7 Process Control System. In addition to the control function all relevant data such as volumes, temperatures, pressures, etc. are recorded and archived in trend charts.

The mash and wort kettle and the newly added adjunct cooker enable all kinds of infusion and decoction mashing methods. Adjuncts such as barley, rice, corn or any other starchy raw materials can even be cooked under pressure. The brewing water comes from wells in the nearby forest.

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The separation of wort and spent grains is carried out either in the lauter tun or the recently built mash filter controlling haze and difference pressure.

With an internal and an external calandria in the mash and wort kettle and a low pressure boiling in the adjunct cooker the pilot plant offers three wort boiling systems. The hops can be prepared in three hop dosing vessels and are flushed with hot wort into the copper at a preselected time.

Hot break is separated in the whirlpool. The cleared wort is cooled in a one stage cooler, aerated by a sinter candle and collected in the pitching tank where yeast is added. Yeast is picked up weekly from a commercial brewery or bought propagated from pure culture from a culture collection.

After the main fermentation in 1.6 hl fermenters and maturation at higher temperature the beer is mellowed for another 2 to 3 weeks at 1 °C in 1.3 hl storage tanks before it is filtered, first through a kieselguhr filter with horizontal plates, and then passed through two PP membrane filters.

A Krones bottling system enables reproducible and oxygen reduced bottling of all kinds of bottles from 0.25 to 0.75 liter. The bottles are pre-evacuated twice and flushed in-between with CO_2. The correction with CO_2 guarantees an equal filling level. If necessary, bottles can be pasteurized in a water bath. Additionally kegs of 10 to 50 liter can be filled on a semi-automatic kegging machine.

2.2 Trial brews

Three brews were made each with the same basic recipe for a highly attenuated pale lager with the following parameters:

- Mash of 17.5 kg Pilsner malt per hectoliter targeting 11.5 °P original extract
- Infusion method with the necessary rests at the following temperatures: 52/64/72/76 (Fig. 1)
- Wort clarification in the lauter tun with 7 spargings
- Wort boiling for 70 minutes using an internal calandria

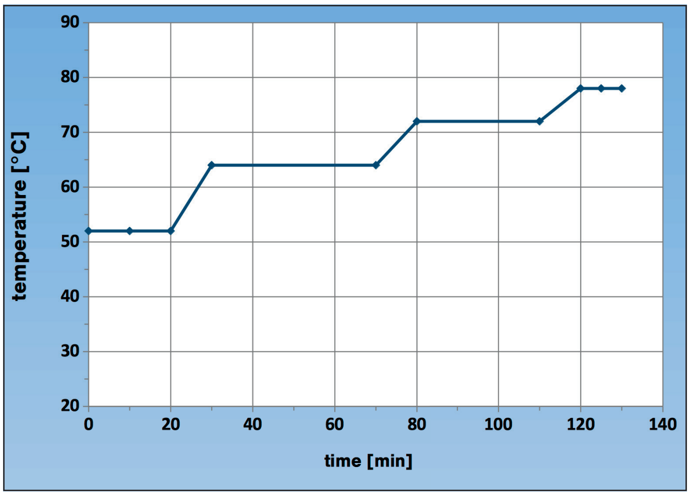

Fig. 1 Infusion mashing method of the brews

- Whirlpool with a rest of 20 minutes and a cooling time of 40 minutes
- Pitching: at 8 °C with a bottom-fermenting W34/70 yeast; first crop from a commercial brewery; pitching rate: 25 million cells/ml
- Main fermentation at 8 °C until reaching a degree of attenuation of > 65 % in 6 days
- Change of tank with maturation at 14 °C for 8 days for diacetyl reduction to <0.08 ppm
- Storage at 0–1 °C for 14 days
- Kieselguhr filtration with a mixture of coarse and fine guhr followed by two membrane filters (pore size: 1.2 and 0.45 µm)
- Bottling in 0.5 l bottles with double pre-evacuation and level correction filling

The three brews were hopped with type 90 pellets of the varieties *Herkules* (HHS) and *Huell Melon* (HHN) both originating from the Hallertau.

Hop dosing in the brewhouse:

- 1st addition at begin of boil: 10 g alpha/hl, variety HHS, 61.75 g pellets/hl
- 2nd addition at end of boil: 150 g/hl, variety HHN
- 3rd addition into the whirlpool: 150 g/hl, variety HHN

The wort was separated into two halves after pitching. One half fermented as is (named block L), the second half has been dry hopped additionally after main fermentation (block D). Dry hopping:

- 150 g/hl pellets of the variety HHN, equal to an oil addition of 1.2 ml/hl
  Pre-addition of pellets at tank change between main fermentation and maturation; contact time: 8 days at 14 °C and 14 days at 0 °C.

2.3 Analysis

2.3.1 Hop analyses

The hop pellets used were analyzed according to the EBC methods 7.7 and 7.10 for determination of the α-acids and total hop oil content respectively:

HHS: α-acids: 16.2 % w/w, total hop oil: 1.70 ml/100 g
HHN: α-acids: 5.5 % w/w, total hop oil: 0.80 ml/100 g

2.3.2 Wort and beer analyses

The wort analyses comprised only a routine check of original extract, final attenuation and pH. The bottled beers were analyzed for the following characteristics:

- Original gravity (OG; % w/w), alcohol by volume (ABV; % v/v), apparent degree of fermentation (ADF; % v) in the laboratory of the research brewery with an Anton Paar Alcolyzer PLUS according to MEBAK "Würze, Bier, Biermischgetränke" 2.9.6.3 [5]
- pH following EBC 9.35 [6]
- Bitterness units according to EBC 9.8 in the laboratory of $Nateco_2$ [6]

Table 2 General beer analyses

	Unit	L 1	L 2	L 3	MEAN	SD_B	D 1	D 2	D 3	MEAN	SD_B
OG	%w/w	11.14	11.28	11.18	11.20	0.07	11.11	11.31	11.25	11.22	0.10
ABV	%vol	4.82	4.89	4.85	4.85	0.04	4.98	5.10	5.08	5.05	0.06
ADF	%	82.5	82.5	82.7	82.6	0.1	85.5	86.0	86.1	85.9	0.3
pH		4.51	4.57	4.56	4.55	0.03	4.50	4.56	4.57	4.54	0.04
CO_2	g/l	5.8	5.7	5.7	5.7	0.1	5.8	5.8	5.7	5.8	0.1

- Iso-α-acids and α-acids as well as xanthohumol (XN) and isoxanthohumol (IX) by HPLC according to EBC 9.47 in the laboratory of $Nateco_2$ [6]
- Total polyphenols according to EBC 9.11 in the laboratory of $Nateco_2$ [6]
- Low-molecular polyphenols determined by HPLC-DAD according to [7] at $Nateco_2$. Determination of the fermentation by-products at KU Leuven using GC-MS as described by *Dresel* et. al. [8].
- Determination of (ageing) aldehydes in beer via HS-SPME with on-fiber PFBHA derivatization in combination with GC-MS by *De Clippeleer* [9], KU Leuven.
- Determination of selected hop aroma components in the bottled beer at KU Leuven via HS-SPME GC-MS [10,11]
- Determination of the R-linalool and S-linalool separated with a chiral column via GC-FID according to [12] at $Nateco_2$.

2.4 Data evaluation

The standard beer analyses were routinely performed as double determinations, all more complex HPLC and GC analyses in triplicate from which the mean values and standard deviations (SD) were calculated. For the evaluation of the reproducibility two different standard deviations are compared:

SD_A is the analytical SD of the three-fold determinations (not shown in the following tables). Since these do not differ substantially the highest SD_A of these analyses ($SD_{A\,max}$) is selected, listed and used for comparison with the standard deviation of the 3 resp. 6 mean values of one or both blocks (SD_B). Wherever suitable the substances of one group of components are added up. The $SD_{A\,max}$ of the individual components are accumulated to a sum of $SD_{A\,max}$ in the tables.

When SD_B is lower than $SD_{A\,max}$ the brews can be considered as reproducible.

2.5 Ageing of the beers

Samples were stored at –24, 0, 20 and 30 °C. The results of these ageing trials will be discussed in part 2.

3 Results

The beers are named as following:

- Block L = only late hopped: L1, L2 and L3
- Block D = additionally dry hopped: D1, D2 and D3

3.1 General beer analyses

Table 2 shows the mean values of each double determination of the 6 brews and the mean values and standard deviations of the two blocks of three brews each. The standard deviations of the brews within the brew blocks (SD_B) is low, which demonstrates the reliability of the plant.

An interesting observation is that the alcohol content and the degree of fermentation are higher in the additionally dry hopped beers (D) than in the beers that were only hot hopped (L). Comparing the two brew blocks the hot hopped beers contained 4.85 % alcohol by volume (ABV) at 82.6 % apparent degree of fermentation (ADF) while the additionally dry hopped beers had 5.05 % ABV at 85.9 % ADF. By dry hopping, fermentable carbohydrates present in hops [13] were obviously introduced after the main fermentation but still with living yeast cells. This will be discussed in more detail later in part 3.

3.2 Bitter substance analyses

With this HPLC method the prenylflavonoids XN and IX are analyzed simultaneously and therefore discussed together with the bitter acids. Table 3 shows the bitter substance analyses of the beers. With the bitter substances $SD_{A\,max}$ and SD_B are at least in a similar range indicating still a good reproducibility within the three beers of a block. Inevitably the $SD_{A\,max}$ are relatively high in particular with substances with a low level like e. g. Xanthohumol.

Between the blocks there are significant differences in the bitterness units (IBU) and α acids (AA). Values are significantly higher in the beers with additional dry hopping which is in accordance

Table 3 Bitter Substance analyses [mg/l]

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
Bittereinheiten (IBU)	27.0	28.0	29.3	0.42	28.1	1.15	30.5	32.0	33.8	0.49	32.9	1.27
Xanthohumol	0.10	0.09	0.09	0.03	0.09	0.01	0.13	0.10	0.17	0.03	0.15	0.03
Isoxanthohumol	0.50	0.56	0.60	0.04	0.55	0.05	0.55	0.53	0.62	0.04	0.60	0.04
Iso-α-acids	24.8	24.3	25.7	0.52	24.9	0.70	24.2	23.8	25.2	0.51	24.8	0.57
α-acids	2.7	2.5	3.5	0.46	2.9	0.51	4.1	4.3	5.5	0.54	5.1	0.64

Table 4 Polyphenol analyses: total polyphenols and low molecular polyphenols (PP) in mg/l ($SD_{A\,max}$ here of all 6 beers)

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	MEAN	SDB
Total Polyphenols	187	182	187	3.0	185.3	2.9	207	210	214	210.3	3.5
Procyanidin B3	0.8	0.7	0.9	0.16	0.8	0.1	1.2	0.9	0.9	1.0	0.2
Caffeeoylquinic acid	0.9	0.9	1.0	0.10	0.9	0.1	1.6	1.8	1.7	1.7	0.1
Catechin	4.8	5.5	5.3	0.26	5.2	0.4	5.7	5.8	5.5	5.7	0.2
Epicatechin	1.5	1.4	1.3	0.19	1.4	0.1	2.0	1.8	1.5	1.8	0.3
Quercetin glucoside	2.4	2.6	2.6	0.09	2.5	0.1	3.9	4.1	3.9	4.0	0.1
Quercetin malonyl hexoside	0.8	0.8	0.9	0.06	0.8	0.1	1.4	1.5	1.4	1.4	0.1
Kaempferol-3-glucoside	1.6	1.6	1.6	0.12	1.6	0.0	2.7	2.8	2.7	2.7	0.1
Kaempferol malonyl hexoside	0.7	0.8	0.8	0.08	0.8	0.1	1.3	1.4	1.4	1.4	0.1
Sum of 8 low molecular PP	13.5	14.3	14.4	1.06	14.1	0.5	19.8	20.1	19.0	19.6	0.6

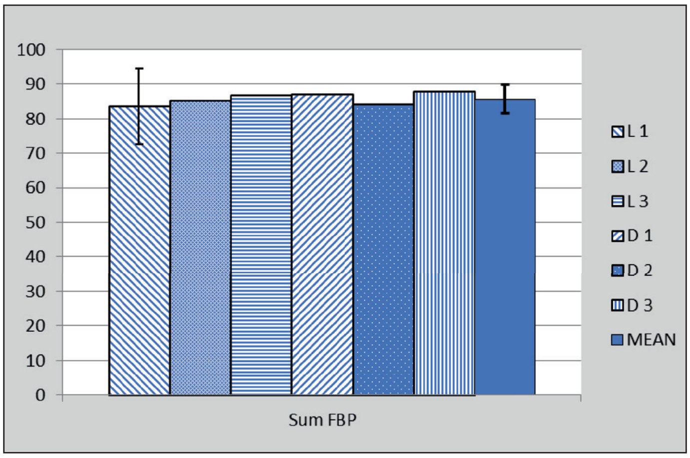

Fig. 2 Sum of fermentation by-products (error bars $SD_{A\,max}$ and SD_B)

with earlier observations [2]. Xanthohumol levels are also higher in the dry hopped beers.

On the other hand, there are no recognizable differences between the brew blocks with regard to the isoxanthohumol and iso-α acid (IAA) contents. Their formation requires a heat holding time.

3.3 Polyphenol analyses

Table 4 shows the polyphenol analyses. The SD_B of the total polyphenol determinations are quite low in the three brews of one block. Another eight single substances, introduced mainly with the hops, were determined by HPLC as described in section 2.3.2. SD_B of these is in the same range as $SD_{A\,max.}$

The total polyphenol levels as well as the levels of six separate substances are significantly higher in the dry hopped beers. It has already been established that polyphenols are soluble with dry hopping [3, 4].

3.4 Determination of the fermentation by-products (FBP)

The content of twelve substances and their sum with $SD_{A\,max}$ and SD_B of all six brews is displayed in table 5. There is a good match between the beers regardless their hopping. This proves that the fermentation was reproducible to a large extent.

Figure 2 shows the sum of the twelve fermentation by-products and clearly their reproducibility.

Table 5 Fermentation by-products [mg/l]

	L 1	L 2	L 3	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
n-propanol	11.7	10.7	12.4	11.4	14.0	14.4	2.9	12.43	1.48
ethyl acetate	17.4	18.3	18.2	18.9	17.3	17.4	2.3	17.92	0.65
isobutanol	8.6	9.5	9.1	9.3	8.1	8.6	2.1	8.87	0.52
3-methylbutanol	35.8	36.1	36.6	36.6	35.1	37.4	2.7	36.27	0.79
2-methylbutanol	8.4	9.1	8.5	9.1	7.9	8.5	0.8	8.56	0.45
isobutyl acetate	0.026	0.023	0.027	0.025	0.025	0.023	0.002	0.025	0.002
ethyl butanoate	0.076	0.085	0.079	0.084	0.068	0.076	0.007	0.078	0.006
isoamyl acetate	0.783	0.737	0.901	0.825	0.801	0.665	0.062	0.785	0.080
ethyl hexanoate	0.124	0.120	0.131	0.116	0.111	0.103	0.007	0.118	0.010
ethyl octanoate	0.189	0.176	0.227	0.197	0.198	0.188	0.018	0.196	0.017
phenylethyl acetate	0.258	0.217	0.323	0.246	0.299	0.163	0.012	0.251	0.057
ethyl decanoate	0.050	0.032	0.061	0.036	0.057	0.037	0.010	0.046	0.012
Sum FBP	**83.4**	**85.1**	**86.6**	**87.0**	**84.0**	**87.6**	**10.9**	**85.6**	**4.1**

Table 6 Ageing aldehydes [µg/l]

	L 1	L 2	L 3	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
2-methylpropanal	6.8	5.2	7.4	7.6	6.2	8.4	0.80	6.93	1.13
2-methylbutanal	1.2	0.8	1.9	0.6	1.5	0.7	0.10	1.12	0.51
3-methylbutanal	4.8	2.0	5.5	1.1	4.6	1.5	0.20	3.25	1.93
methional	3.3	3.8	5.8	2.6	4.0	2.5	1.00	3.67	1.21
phenylacetaldehyde	8.3	5.2	8.6	5.3	7.9	3.6	1.30	6.48	2.06
benzaldehyde	1.0	1.0	1.0	0.9	0.9	1.0	0.05	0.96	0.05
pentanal	0.16	0.17	0.18	0.14	0.14	0.15	0.08	0.16	0.02
t-2-pentenal	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.02	0.00
hexanal	0.094	0.070	0.093	0.063	0.084	0.069	0.006	0.079	0.013
t-2-hexenal	0.0	0.1	0.0	0.1	0.0	0.1	0.01	0.05	0.01
heptanal	0.16	0.15	0.14	0.10	0.14	0.12	0.02	0.14	0.02
t,t-2,4-heptadienal	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.03	0.00
octanal	0.13	0.15	0.18	0.16	0.14	0.12	0.02	0.15	0.02
t-2-octenal	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.02	0.00
t,t-2,4-octadienal	0.015	0.011	0.011	0.012	0.012	0.016	0.005	0.013	0.002
nonanal	0.3	0.4	0.5	0.3	0.3	0.3	0.09	0.33	0.09
t-2-nonenal	0.014	0.013	0.015	0.013	0.012	0.014	0.002	0.014	0.001
decanal	0.4	0.4	0.4	0.4	0.4	0.3	0.09	0.38	0.04
furfural	7.9	9.2	8.0	9.9	6.3	8.1	1.10	8.23	1.24
Sum Aldehydes	**34.7**	**28.7**	**39.8**	**29.2**	**32.7**	**27.0**	**4.89**	**32.01**	**8.34**

3.5 Aldehydes

Table 6 shows 19 (ageing) aldehydes. In particular there are minimal differences between the hot hopped and dry hopped beers. Therefore it is not useful to observe them separately. The SD_A are in an acceptable range taken the low values in fresh beers which corresponds with the published values of De Clippeleer [9]. The low absolute levels are much below reported threshold values. A consideration of SD_A and SD_B makes more sense in the aged beers and of course a comparison of the aged vs. the fresh beers. Next to the reproducibility aspect, the impact of dry hopping on the appearance of (unwanted) aldehydes could also be evaluated in this ongoing study (Part 2).

Looking at the total aldehyde levels (Fig. 3) brew L 3 steps somewhat out of line, the fluctuations in the remaining 5 brews are moderate. This has to be considered when evaluating the aged beers.

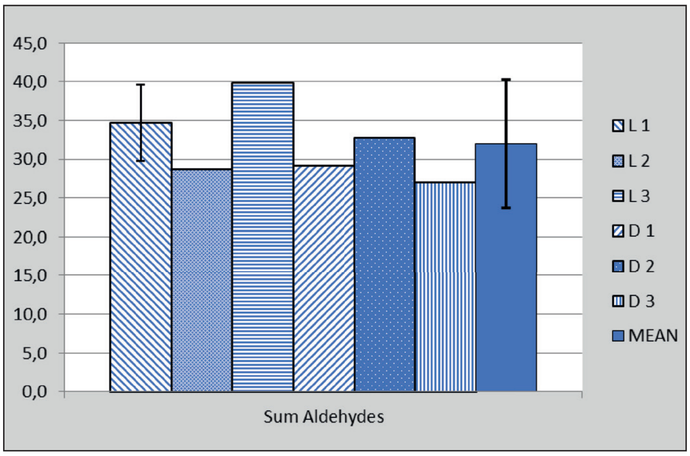

Fig. 3 Total aldehyde levels

3.6 Hop aroma substances

The aroma substances selected were those that were detected in beers that were only hot hopped. It should be noted that the particularly volatile monoterpenes (e.g. β-myrcene) and sesquiterpenes that dissolve with difficulty in beer (e. g. α-humulene), can only survive the heat holding time in traces. Altogether 5 monoterpenes and 15 sesquiterpenes were analyzed and grouped. The terpene

Table 7 Monoterpene hydrocarbons (MTH) [µg/l]

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
β-myrcene	4.19	3.52	4.69	0.06	4.13	0.59	6.23	5.45	5.05	0.36	5.58	0.60
limonene	1.35	1.26	1.31	0.02	1.31	0.05	2.31	2.35	2.23	0.05	2.30	0.06
cis-ocimene	0.09	0.08	0.11	0.04	0.09	0.02	0.21	0.19	0.19	0.03	0.20	0.01
trans-ocimene	0.67	0.59	0.83	0.06	0.70	0.12	1.37	1.39	1.32	0.05	1.36	0.04
terpinolene	0.12	0.10	0.11	0.02	0.11	0.01	0.20	0.19	0.18	0.03	0.19	0.01
Sum MTH	**6.42**	**5.55**	**7.05**	**0.2**	**6.34**	**0.78**	**10.33**	**9.56**	**8.97**	**0.52**	**9.62**	**0.72**

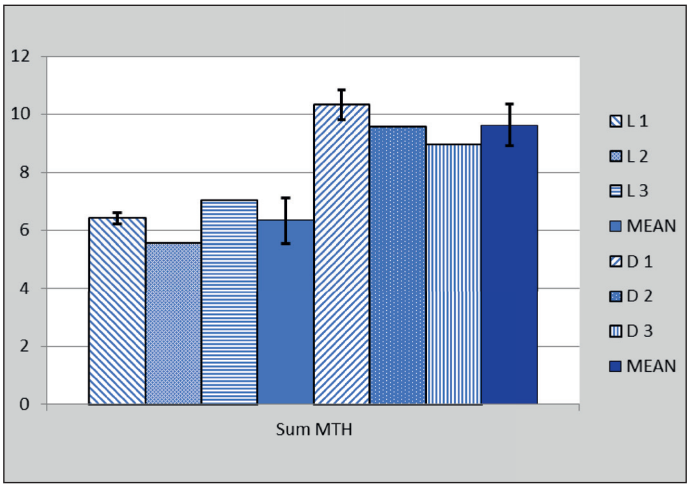

Fig. 4 Total level of monoterpene hydrocarbons

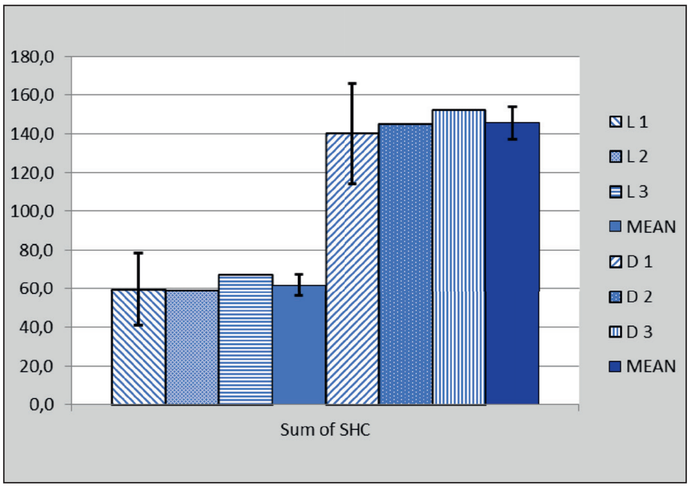

Fig. 5 Total level of sesquiterpene hydrocarbons

levels are admittedly low due to intensified precipitation processes in a pilot brewery of small size.

The reproducibility of the system can be better examined with more polar, oxygenated substances which dissolve more readily in wort and beer. In the present case we report the level of 12 carboxylic esters, 4 monoterpene alcohols, 11 sesquiterpene alcohols and 3 epoxides.

3.6.1 Monoterpenes

The mean values, $SD_{A\,max}$ and SD_B of the monoterpenes are given in Table 7. The fluctuations of the analyses are surprisingly low considering their volatility. In contrast, the fluctuations in the brew blocks are more distinctive. With dry hopping the sum of monoterpenes increases from 6.3 to 9.6 µg/l, or by 52 %. This can be seen in figure 4.

Compared to the low analytical error bars the differences between the brews appear rather large. In former trials the SD_A especially of myrcene was significantly higher and in a range of 2 µg/l at similar absolute values. Furthermore it is not reasonable to discuss trace levels far below thresholds.

3.6.2 Sesquiterpenes

Table 8 gives the content of 15 different components along with their standard deviations. In contrast to the monoterpenes the analytical dispersions are much more distinctive, which is less surprising. The 3 brews in both blocks match well contrary to the monoterpenes ($SD_B < SD_{A\,max}$).

Taking the sum of the sesquiterpenes, figure 5 verifies the good reproducibility of the brews. Dry hopping increases the total levels from 62 to 146 µg/l, which is 2.4-fold. Apparently, β-caryophyllene, farnesene and humulene, the major sesquiterpene hydrocarbons

Table 8 Sesquiterpene hydrocarbons (SHC) in µg/l

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
β-caryophyllene	1.14	1.16	1.21	0.22	1.17	0.04	1.12	1.02	1.02	0.18	1.05	0.06
α-humulene	5.35	4.89	5.13	0.89	5.12	0.23	4.86	4.41	4.00	0.73	4.42	0.43
farnesene	1.98	1.85	2.15	0.64	1.99	0.15	1.65	1.36	1.72	0.33	1.58	0.19
α-elemene	1.77	1.89	2.15	0.71	1.94	0.19	4.42	5.02	5.76	1.68	5.07	0.67
γ-muurolene	10.41	10.68	11.38	3.04	10.82	0.50	25.88	27.22	27.89	4.49	27.00	1.02
β-selinene	10.37	10.79	10.34	2.86	10.50	0.25	28.78	29.80	31.80	4.42	30.13	1.54
δ-selinene	2.86	3.01	3.12	0.92	3.00	0.13	8.84	9.49	11.09	2.41	9.81	1.16
α-selinene	13.34	13.84	13.92	4.22	13.70	0.31	37.11	38.44	39.74	5.98	38.43	1.32
γ-cadinene	1.15	1.05	2.73	0.41	1.64	0.94	2.21	2.19	2.71	0.29	2.37	0.29
***trans*-calamenene**	1.37	1.21	2.10	0.38	1.56	0.47	2.29	2.12	2.08	0.40	2.16	0.11
δ-cadinene	2.35	1.99	4.35	0.48	2.90	1.27	3.92	3.97	4.77	0.95	4.22	0.48
SHC	1.32	1.13	1.23	0.49	1.23	0.10	2.16	2.32	2.55	0.72	2.34	0.20
SHC	1.79	2.11	1.87	0.53	1.92	0.17	5.17	5.64	5.54	0.95	5.45	0.25
α-calacorene	1.80	1.74	2.25	0.61	1.93	0.28	4.38	4.28	3.82	0.94	4.16	0.30
selina-3,7(11)-diene	2.61	1.83	2.95	2.29	2.46	0.57	7.30	7.91	8.02	1.47	7.74	0.39
Sum of SHC	**59.6**	**59.1**	**66.9**	**18.7**	**61.9**	**5.6**	**140.0**	**145.0**	**152.0**	**25.9**	**145.7**	**8.4**

Table 9 Carboxylic esters [µg/l]

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
isobutyl isobutyrate	48.9	45.7	49.9	4.4	48.2	2.2	153.8	168.6	152.0	7.8	158.1	9.1
butyl isobutyrate	1.36	1.17	1.17	0.32	1.23	0.11	4.18	4.54	4.23	0.25	4.32	0.20
2-methylbutyl propanoate	5.00	6.49	6.52	0.36	6.00	0.87	13.20	13.86	12.34	0.81	13.13	0.76
3-methylbutyl 2-methylpropanoate	30.1	28.5	32.5	2.9	30.4	2.0	104.2	109.9	108.1	5.9	107.4	2.9
2-methylbutyl 2-methylpropanoate	338.3	326.5	392.2	10.8	352.3	35.0	1062.2	1117.9	1109.7	31.8	1096.6	30.1
methyl 4-methylenehexanoate	7.72	8.00	8.20	0.37	7.97	0.25	18.62	19.84	19.48	0.96	19.31	0.63
ethyl 4-methylnonanoate	0.26	0.29	0.29	0.04	0.28	0.01	0.51	0.69	0.65	0.19	0.62	0.09
2-methylbutyl 3-methylbutanoate	5.08	5.95	8.09	1.43	6.37	1.55	21.94	21.48	25.92	0.55	23.11	2.44
2-methylbutyl 2-methylbutanoate	3.88	4.66	6.14	1.21	4.89	1.15	15.78	15.12	18.89	0.79	16.60	2.02
unidentified ethyl ester	16.0	18.2	18.5	4.1	17.6	1.4	29.2	35.0	36.7	8.7	33.6	4.0
ethyl 4-methyloctanoate	1.52	1.94	1.90	0.76	1.78	0.23	2.81	3.48	3.83	1.42	3.37	0.52
ethyl trans-4-decenoate	3.84	4.61	4.39	1.91	4.28	0.40	6.96	7.60	8.06	2.40	7.54	0.55
Sum of carboxylic esters	**462.1**	**452.0**	**529.8**	**28.7**	**481.3**	**45.2**	**1433.4**	**1518.0**	**1499.9**	**61.5**	**1483.8**	**53.2**

in the hop essential oil are not part of this increase, the selinenes and ɣ-muurolene have the highest augmentation. This observation cannot be explained at the moment.

3.6.3 Carboxylic esters

Table 9 shows 12 substances from the group of carboxylic esters accordingly. In most of the cases SD_A is lower than SD_B. There are negligible differences between the brews. Only brew L3 stands out slightly mainly because of the 2-methylbutyl 2-methylpropanoate which influences also the sum remarkably.

The sum of the 12 esters are shown in figure 6. The blocks match well. On average in the brews the esters go from 481 µg/l in the hot hopped beers to 1,484 µg/l in the dry hopped beers, a 3.1-fold increase. Striking is the fact that the 3 ethyl esters increase only 1.9-fold with dry hopping. Probably they are (partially) transformed from methyl esters to ethyl esters during fermentation.

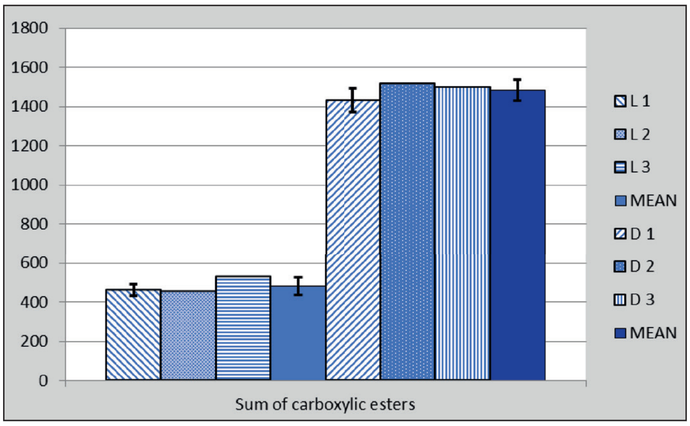

Fig. 6 Total level of the hop related esters

3.6.4 Monoterpene alcohols

Table 10 deals with the 4 monoterpene alcohols in a meanwhile familiar manner. The fluctuations of the analyses are surprisingly low, only in a few cases SD_B is higher than SD_A but in a similar range. The nerol and geraniol values were all under the detection limit (10 µg/L).

Figure 7 shows the comparisons of the sum of the monoterpene alcohols. Brew L1 stands out with a slightly elevated value. Nevertheless, the brews display satisfactory standard deviations. Dry

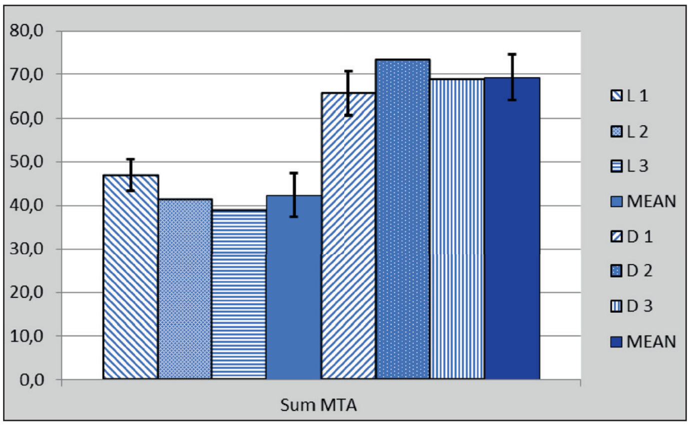

Fig. 7 Total level of monoterpene alcohols (MTA)

Table 10 Monoterpene alcohols [µg/l]

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
linalool	25.6	19.8	17.1	2.1	20.8	4.3	32.2	37.6	33.5	3.0	34.4	2.8
p-menth-1-en-4-ol	2.26	2.30	2.20	0.10	2.25	0.05	3.20	3.20	3.00	0.10	3.13	0.12
α-terpineol	5.01	5.50	5.70	0.30	5.40	0.36	10.70	11.00	9.50	1.10	10.40	0.79
citronellol	30.1	28.5	32.5	2.9	30.4	2.0	104.2	109.9	108.1	5.9	107.4	2.9
Sum MTA	**47.0**	**41.4**	**38.7**	**3.6**	**42.4**	**5.0**	**65.7**	**73.3**	**68.9**	**5.0**	**69.3**	**5.2**

Table 11 R-linalool ratio [% rel.]

	L 1	L 2	L 3	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
R-Linalool ratio	**94.1**	**92.9**	**87.5**	**90.0**	**90.6**	**87.1**	**2.5**	**90.4**	**2.8**

hopping increases the monoterpene alcohols by 1.5 times, which is less than with other substance groups.

3.6.5 R-linalool and S-linalool

The ratio of R-linalool in % rel. of the total linalool was calculated and described as an indicator for the ageing character of the beers and the hops used for their production [12]. Values around 90 % are normal for fresh hops and beers. Table 11 gives the mean values of the R-linalool ratio, $SD_{A\,max}$ and SD_B in all 6 beers and shows no significant differences between late and dry hopped beers. $SD_{A\,max}$ and SD_B are almost the same.

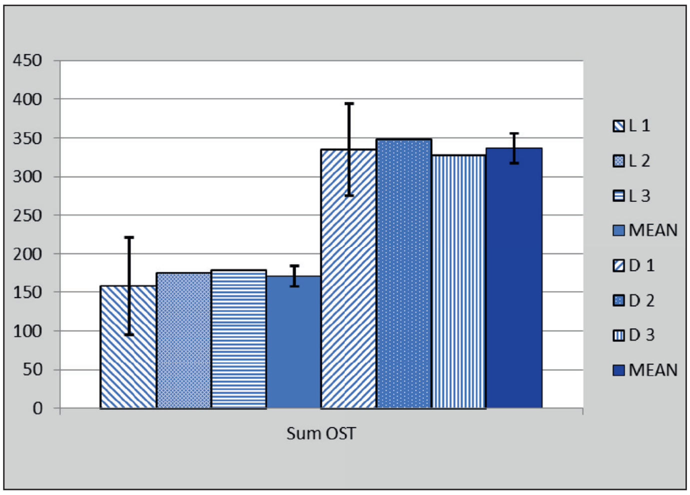

Fig. 8 Total level of oxygenated sesquiterpenoids [µg/l]

3.6.6 Oxygenated sesquiterpenoids

Of the total of 14 analyzed components we are reporting the levels of 3 epoxides and 11 alcohols in table 12 SD_A is always higher than SD_B which indicates a reproducible brewing setup. The 11 sesquiterpene alcohols increase from 123 to 295 µg/l significantly (2.4-fold) through dry hopping. whereas the level of the major epoxide (caryophyllene oxide) didn't increase. This observation is in line with the findings of *Praet* et. al. [14] that epoxides are mainly formed as a result of the boiling process (e.g. transformation of β-caryophyllene and α-humulene into oxygenated sesquiterpenoids), whereas sesquiterpene alcohols could originally be present in significant amounts (depending on the hop variety) in the hop oil (hop pellets) as such as a result of the plant metabolism. This explains why dry hopping, as observed in our trials, does not result in an increase in the sesquiterpene epoxide level.

Figure 8 shows the sums of the 15 oxygenated sesquiterpenoids in the 6 brews. The brews in the blocks are very similar to each other. The mean of the hot hopped beers is 175 µg/l compared to 340 µg/l with the dry hopped beers.

4 Summary

Due to costs, trial brews usually involve just single brews in most studies. Our research brewery has reproducibility data on the complete beer production process based only on the general beer analyses like OG, ABV, pH, CO_2, color, IBU and total polyphenols. To date the formation of fermentation by-products and aging aldehydes has been neglected. Even hop-specific

Table 12 Oxygenated sesquiterpenoids (OST)

	L 1	L 2	L 3	$SD_{A\,max}$	MEAN	SD_B	D 1	D 2	D 3	$SD_{A\,max}$	MEAN	SD_B
***trans*-nerolidol**	3.27	2.76	3.00	0.56	3.01	0.26	6.07	5.22	4.05	0.95	5.11	1.01
caryophyllene oxide	36.7	43.5	45.7	15.7	41.9	4.7	34.0	42.4	37.5	20.1	38.0	4.2
humulene epoxide I	7.74	7.06	12.02	2.89	8.94	2.69	3.46	3.48	3.47	0.55	3.47	0.01
humulene epoxide II	0.47	0.23	0.42	0.15	0.37	0.13	0.57	0.56	0.50	0.11	0.54	0.04
juniper camphor	16.7	15.3	14.5	2.2	15.5	1.1	46.7	45.0	38.3	8.1	43.3	4.4
cubenol	3.56	2.85	2.87	0.30	3.09	0.40	6.40	5.52	4.48	0.99	5.47	0.96
sesquiterpene alcohols	12.9	12.4	11.3	1.8	12.2	0.8	28.5	29.1	29.3	7.4	28.9	0.4
γ-eudesmol	12.4	13.6	12.4	4.7	12.8	0.7	35.0	36.4	34.3	2.2	35.2	1.1
τ-cadinol	26.9	27.2	27.7	20.3	27.2	0.4	61.2	61.7	64.8	6.7	62.6	1.9
cubenol	4.73	4.86	5.17	1.20	4.92	0.23	8.62	9.13	7.96	2.43	8.57	0.59
β-eudesmol	4.70	4.98	4.96	1.73	4.88	0.16	13.07	13.66	13.07	0.67	13.27	0.34
α-cadinol	11.2	11.1	10.3	3.9	10.9	0.5	25.7	28.0	25.4	2.0	26.4	1.4
α-eudesmol	24.7	26.2	25.1	6.6	25.3	0.7	58.7	60.6	56.5	4.3	58.6	2.0
eudesmol-like	2.22	2.73	3.02	0.53	2.66	0.41	6.94	7.18	8.12	2.58	7.41	0.62
Sum OST	**168.2**	**174.7**	**178.4**	**62.6**	**173.8**	**13.3**	**334.9**	**347.7**	**327.7**	**59.1**	**336.8**	**19.1**

characteristics like low-molecular polyphenols, and in particular hop aroma substances in beer, have not been considered with regard to reproducibility yet.

The increasing significance of hops for many beers, especially in the craft beer segment, is leading to more trial brews focussing on hops. Here it is obligatory to examine the reliability of a trial plants design. For this reason reproducibility brews were made with the following characteristics:

- 3 identical brews with a hop addition at begin of boil and at end of boil
- The brews were divided as follows after the main fermentation:
  - one half was bottled as late hopped beers (block L)
  - the other half was additionally dry hopped before maturation at tank change and bottled separately (block D)

All beers were analyzed for the following properties, sensory evaluations were not employed:

- General beer characteristics
- Bitter substances (IBU and HPLC)
- Polyphenols (total and 8 low-molecular polyphenols using HPLC)
- 12 fermentation by-products (GC)
- 19 (aging) aldehydes (GC)
- 50 hop aroma substances (GC)

In addition to the 3-fold determinations of the HPLC and GC analyses the analytical standard deviations (SD_A) were established alongside the mean values. Furthermore, of the 3 beers of each block the mean value and standard deviation of the three beers (SD_B) was calculated. For a critical SD_A assessment the maximum SD_A of a 3-series or of a 6-series respectively was taken. A direct comparison of the analytical standard deviations ($SD_{A\,max}$) with the beer production standard deviations (SD_B) gives a measure of the accuracy of the trial plant.

Altogether 185 comparisons of analytical and production-related standard deviations (SD) were made. In 65 cases the SD_B is higher than the $SD_{A\,max}$ in purely arithmetical terms. However, these are only slight differences and mostly in analytical features that are found at trace level in the beers. This concerns in particular the monoterpenes and the aging aldehydes (freshly bottled beers). Discussions about trace values are problematic anyway.

The good reproducibility of the complete beer production process in the research brewery is proven not only by standard analyses but also by the fermentation by-products and the aging aldehydes. In particular, however, the hop-related and difficult analyses of the low-molecular polyphenols and the aroma components show no or only slight production-related deviations in the beers. The design of the research brewery is well suitable for examining the influence of hopping parameters on beer components. Backed up with this study carefully performed single brews therefore are qualified to evaluate hopping parameters.

In a continuing paper the analytical results and the reproducibility regarding the aged beers will be examined and some technological findings from the trials will be described.

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