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

Part 2: Reproducibility in Moderately Aged Beers

The good reproducibility of the 2 hl research brewery as proven in our previous study has been now reviewed after storing the beers at 0 °C and deep frozen (at – 24 °C) for 240 days. Except for some Strecker aldehydes all other ageing aldehydes, fermentation by-products and particularly the analyzed hop aroma compounds show good stability at 0 °C. The analytical standard deviations of the three-fold determinations are even a little lower than those of the fresh beers. Also the spread (standard deviations) of the results of the repeated brews did not increase. Opposite to that some hop aroma characteristics of the deep frozen dry hopped beers differed significantly from the ones of the fresh beers. On the basis of these findings this kind of sample preservation for considerably hopped beers cannot be recommended.

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

1 Introduction

In the first installment in this series, the reproducibility of brewing trials in a pilot brewery with a capacity of 2 hl (cast-out wort) was discussed [1]. The primary focus of the analysis was on substances derived from hops, fermentation by-products and the aldehydes associated with ageing. Three-fold determinations were performed for each measurement in these trials. In each, three late-hopped beers were brewed according to the same recipe, as were three dry-hopped beers. This recipe served as the basis for all of the beer in these trials. If the standard deviation of one of the measured attributes of the three beers was equal to or below the standard deviation of the three-fold determination, the production-related range of variance was thus similar to or less than the pure analytical range of variance. If this turned out to be the case, production of the beer was deemed reproducible. In summary, the result was assessed as follows: "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 ageing 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.

The second part of this investigation examined whether cold ageing over a moderate duration brings about changes in beer with regard to the absolute values of the measured parameters, the values for the analytical standard deviation and the standard deviation of the multiple brews. Information on this subject is rather scarce.

At the EBC Congress in 2017, *Biendl* reported on the extreme variation in the analysis of hop aroma compounds in beer in a comparison across various laboratories [2]. The pursuit of a reliable and in ring analyses approved method of analysis is apparently far from over. Calibrating laboratory instruments, for example, for the purpose of measuring myrcene and linalool is still fraught with difficulties, and thus a comparison of the results from several laboratories is practically considered out of the question. But it is also important to examine the repeatability within a laboratory. If the hop aroma in beer is stable for a prolonged period when stored at cold temperatures (0 °C) or even deep frozen (– 24 °C), samples

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Table 1 Fermentation By-products (FBP); average of late and dry [mg/l]; fresh = 6 beers, 0 °C = 4 beers, – 24 °C = 2 beers

	MEAN fresh	MEAN 0 °C	MEAN – 24 °C	$SD_{A\,max}$ fresh	$SD_{A\,max}$ 0 °C	$SD_{A\,max}$ – 24 °C	SD_B fresh	SD_B 0 °C
n-propanol	12.43	11.00	8.90	2.90	3.70	1.00	1.48	0.90
ethyl acetate	17.92	17.00	10.30	2.30	2.00	0.10	0.65	1.60
isobutanol	8.87	8.90	6.30	2.10	0.90	0.30	0.52	0.40
3-methylbutanol	36.27	37.00	34.90	2.70	3.50	0.60	0.79	2.40
2-methylbutanol	8.56	8.50	8.60	0.80	0.80	0.15	0.45	0.80
isobutyl acetate	0.025	0.025	0.025	0.002	0.004	0.001	0.002	0.002
ethyl butanoate	0.078	0.078	0.082	0.007	0.010	0.002	0.006	0.100
isoamyl acetate	0.785	0.783	0.850	0.062	0.117	0.016	0.080	0.040
ethyl hexanoate	0.118	0.122	0.110	0.007	0.014	0.006	0.010	0.030
ethyl octanoate	0.196	0.208	0.190	0.018	0.023	0.039	0.017	0.100
phenylethyl acetate	0.251	0.193	0.210	0.012	0.002	0.001	0.057	0.060
ethyl decanoate	0.046	0.049	0.050	0.010	0.012	0.030	0.012	0.030
Sum FBP	**85.5**	**83.9**	**70.5**	**10.9**	**11.1**	**2.2**	**4.1**	**6.5**

Table 2 Ageing aldehydes; average of late and dry [µg/l]; fresh = 6 beers, 0 °C = 4 beers, -–24 °C = 2 beers

	MEAN fresh	MEAN 0 °C	MEAN – 24 °C	$SD_{A\,max}$ fresh	$SD_{A\,max}$ 0 °C	$SD_{A\,max}$ – 24 °C	SD_B fresh	SD_B 0 °C
2-methylpropanal (S)	6.9	14.3	8.3	0.8	1.1	0.4	1.1	7.7
2-methylbutanal (S)	1.1	2.1	3.9	0.1	0.1	0.1	0.5	0.9
3-methylbutanal (S)	3.3	7.1	6.1	0.2	0.3	0.2	1.9	2.7
methional (S)	3.7	4.1	2.6	1.0	1.1	0.3	1.2	2.1
phenylacetaldehyde (S)	6.5	7.5	3.9	1.3	1.3	0.9	2.1	3.5
benzaldehyde (S)	0.96	0.95	0.73	0.05	0.04	0.04	0.05	0.07
pentanal (F)	0.160	0.160	0.160	0.080	0.080	0.010	0.020	0.030
t-2-pentenal (F)	0.020	0.022	0.014	0.010	0.007	0.002	0.000	0.003
hexanal (F)	0.079	0.079	0.110	0.006	0.006	0.007	0.013	0.013
t-2-hexenal (F)	0.050	0.048	0.032	0.010	0.007	0.005	0.010	0.007
heptanal (F)	0.140	0.140	0.150	0.020	0.020	0.030	0.020	0.030
t, t-2,4-heptadienal (F)	0.030	0.021	0.035	0.010	0.008	0.006	0.000	0.005
octanal (F)	0.150	0.140	0.180	0.020	0.020	0.040	0.020	0.030
t-2-octenal (F)	0.020	0.021	0.025	0.000	0.003	0.004	0.000	0.004
t, t-2,4-octadienal (F)	0.013	0.013	0.013	0.005	0.005	0.010	0.002	0.002
nonanal (F)	0.330	0.330	0.320	0.090	0.090	0.320	0.090	0.100
t-2-nonenal (F)	0.014	0.016	0.019	0.002	0.002	0.004	0.001	0.001
decanal (F)	0.380	0.360	0.280	0.090	0.090	0.050	0.040	0.002
furfural (M)	8.2	8.6	3.2	1.1	1.1	0.3	1.2	1.4
Sum ageing aldehydes	**32.0**	**46.0**	**30.1**	**4.9**	**5.4**	**2.7**	**8.4**	**18.6**

collected from months of experimental trials could be kept and analyzed in a single run of tests. This would avoid errors arising from determinations over longer periods.

2 Materials and Methods

A description of the organization of the brewing trials is provided in detail in the first installment of this series [1]. Three identical bottom-fermented beers with original gravities of approximately 11.5 % were hopped as follows:

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 in whirlpool: 150 g/hl, variety HHN.

The wort was separated into two halves after pitching. One half fermented as is, the second half has been dry hopped additionally after main fermentation.

Table 3 Monoterpene Hydrocarbons (MTH) [µg/l]

	MEAN late			MEAN dry			$SD_{A\,max}$ late		$SD_{A\,max}$ dry		SD_B late		SD_B dry	
	fresh	0 °C	– 24 °C	fresh	0 °C	– 24 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C
β-myrcene	4.13	4.46	4.70	5.58	5.06	4.70	0.06	0.57	0.36	0.29	0.59	0.25	0.60	0.03
limonene	1.31	1.28	0.82	2.30	2.15	1.23	0.02	0.13	0.05	0.11	0.05	0.10	0.06	0.18
cis-ocimene	0.09	0.06	0.06	0.20	0.12	0.07	0.04	0.01	0.03	0.04	0.02	0.01	0.01	0.01
trans-ocimene	0.70	0.47	0.51	1.36	0.90	0.56	0.06	0.04	0.05	0.07	0.12	0.04	0.04	0.06
terpinolene	0.11	0.13	0.07	0.19	0.16	0.12	0.02	0.02	0.03	0.02	0.01	0.03	0.01	0.00
Sum MTH	**6.34**	**6.40**	**6.16**	**9.63**	**8.39**	**6.68**	**0.20**	**0.77**	**0.52**	**0.53**	**0.79**	**0.43**	**0.72**	**0.28**

Table 4 Sesquiterpene Hydrocarbons (SHC) [µg/l]

	Mean late			Mean dry			$SD_{A\,max}$ late		$SD_{A\,max}$ dry		SD_B late		SD_B dry	
	fresh	0 °C	– 24 °C	fresh	0 °C	– 24 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C
β-caryophyllene	1.17	1.09	1.94	1.05	0.69	0.74	0.22	0.09	0.18	0.06	0.04	0.32	0.06	0.04
α-humulene	5.12	4.68	6.51	4.42	2.77	3.43	0.89	0.86	0.73	0.40	0.23	0.78	0.43	0.37
β-farnesene	1.99	1.10	2.25	1.58	1.50	1.81	0.64	0.27	0.33	0.30	0.15	0.20	0.19	0.18
α-elemene	1.94	2.58	2.89	5.07	1.67	3.69	0.71	0.45	1.68	1.15	0.19	0.40	0.67	0.11
γ-muurolene	10.82	15.90	16.00	27.00	25.70	18.90	3.04	1.30	4.49	3.80	0.50	0.42	1.02	0.92
β-selinene	10.50	9.10	9.10	30.13	32.4	23.70	2.86	1.60	4.42	4.50	0.25	0.78	1.54	0.42
δ-selinene	3.00	2.02	3.30	9.81	6.02	4.91	0.92	0.42	2.41	1.24	0.13	0.58	1.16	0.43
α-selinene	13.70	13.20	13.50	38.43	39.80	30.30	4.22	2.90	5.98	6.30	0.31	2.05	1.32	0.99
γ-cadinene	1.64	1.35	1.92	2.37	1.86	2.33	0.41	0.34	0.29	0.45	0.94	0.05	0.29	0.05
trans-calamenene	1.56	0.84	1.24	2.16	1.05	1.34	0.38	0.02	0.40	0.08	0.47	0.06	0.11	0.08
δ-cadinene	2.90	1.97	3.29	4.22	2.62	2.73	0.48	0.02	0.95	0.63	1.27	0.05	0.48	0.02
unidentified	1.23	2.71	2.49	2.34	2.75	1.88	0.49	0.50	0.72	0.53	0.10	0.23	0.20	0.18
unidentified	1.92	3.99	3.74	5.45	6.73	4.48	0.53	0.25	0.95	1.09	0.17	0.04	0.25	0.15
α-calacorene	1.93	1.68	2.35	4.16	2.40	1.96	0.61	0.06	0.94	0.27	0.28	0.12	0.30	0.39
selina-3,7(11)-diene	2.46	5.35	5.54	7.74	9.61	7.21	2.29	0.65	1.47	1.38	0.57	0.42	0.39	0.13
Sum SHC	**61.9**	**67.6**	**76.1**	**145.9**	**137.6**	**109.4**	**18.7**	**9.7**	**25.9**	**22.2**	**5.6**	**6.5**	**8.4**	**4.5**

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.

Of these sets of six beers, two late-hopped and two additionally dry hopped beers were stored at 0 °C. One set of each was deep frozen for 240 days at – 24 °C. The analysis was limited to the more delicate volatile aroma compounds in the form of fermentation by-products, aldehydes involved in ageing and hop aroma compounds:

- Determination of the fermentation by-products at KU Leuven using GC-MS as described by *Dresel* et al. [3].
- Determination of (ageing) aldehydes in beer via HS-SPME with on-fiber PFBHA derivatization in combination with GC-MS by *De Clippeleer* [4], KU Leuven.
- Determination of selected hop aroma components in the bottled beer at KU Leuven via HS-SPME GC-MS [5, 6].

GC analyses were performed 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, also after ageing.

A low oxygen bottling is a basic prerequisite for a reproducible beer production. In the research brewery a Krones (system VKPV-CF) semi-automatic bottling device with double pre-evacuation and CO_2-correction is used. The TPO (total packed oxygen) levels in the bottled beers are checked regularly. In the course of the reproducibility trials TPO measurements were performed with an

Orbisphere 6110 Total Package Analyser. With 15 individual results the following performance data (mean ± confidence interval; P = 95 %) could be evaluated:

- TPO mean = 41.0 ± 2.9 ppb with a standard deviation of 5.2 ppb;
- Filling volume mean = 329.3 ± 0.7 ml with a standard deviation of 1.2 ml.

Furthermore it was of great interest whether differences regarding the ageing aldehydes between the 6 brews (3 x late, 3 x dry) could be assessed when ageing the beers moderately. If there were this would indicate a non-optimal bottling and the risk of variations in the TPO of the bottled beers.

3 Results and Discussion

The tables contain the following information:

- Mean values for the fresh samples as well as those aged at 0 °C and – 24 °C (MEAN)
- Values for the maximum standard deviation for the three-fold determinations of the fresh and aged beers ($SD_{A\ max}$). Since the $SD_{A\ max}$ data for the hop aroma compounds in the samples stored at – 24 °C deviated only slightly from those of the samples stored at 0 °C, the list of $SD_{A\ max}$ values for the – 24 °C samples was omitted.
- Values for the standard deviation for the multiple brews (SD_B) of fresh beer and those stored at 0 °C

No distinction was made between the fermentation by-products and the ageing carbonyls in the late-hopped and dry-hopped beers.

3.1 Fermentation By-Products

The following major observations can be made upon reviewing the data on fermentation by-products presented in table 1:

- There are no significant differences between the fresh beers and those stored at 0 °C. The mean values for the individual compounds as well as values for the standard deviation (SD_{Amax}) are highly comparable. Additionally, the values for the standard deviation for the multiple brews (SD_B) are comparable and are also lower overall than the $SD_{A\ max}$ values. The cold storage effected no change in the fermentation by-products.
- The samples stored in the deep freeze exhibited lower than average values and lay outside the values for the analytical standard deviation for n-propanol, isobutanol and especially for ethyl acetate. A credible explanation has yet to be found for this observation. One should note that freezing may bring about changes to the structure of beer and may prevent reliable measurement values from being obtained.

3.2 Aldehydes Associated with Ageing

The ageing carbonyls grouped as Strecker aldehydes (S), fatty acid oxidation products (F) and Maillard reaction substance (M) in table 2 show only for the 0 °C beers a discernible trend. All individual Strecker aldehydes and the sum of the 0 °C samples (36.1 µg/l) exceed the data for the fresh beers (sum: 22.4 µg/). The beers stored in the freezer do not show a consistent behavior. Even some of the aldehydes (methional, phenylacetaldehyde and furfural) show lower figures than in fresh beers. So freezing of the samples does not lead to the aimed sample preservation even in case of ageing aldehydes.

In the fresh and aged samples, the values for the analytical standard deviation ($SD_{A\ max}$) are similar. However, there is a clear difference in the SD_B of individual samples of fresh beer and those stored at 0 °C, e.g 2-methylpropanal (7.7 µg/l). The concentrations of 2-methylpropanal are present at significantly lower levels (9.9 µg/l) in late-hopped beer than in the dry-hopped beer (17.8 µg/l). This phenomenon will be the subject of further investigation in ageing trials.

The standard deviations of all components of the late and the dry hopped beers were at the same level as the fresh beers indicating a reproducible bottling procedure.

Table 5 Hop Esters [µg/l]

	Mean late			Mean dry			$SD_{A\ max}$ late		$SD_{A\ max}$ dry		SD_B late		SD_B dry	
	fresh	0 °C	– 24 °C	fresh	0 °C	– 24 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C	fresh	0 °C
isobutyl isobutyrate	48.2	53.6	59.0	158.1	165.5	91.9	4.4	2.2	7.8	5.0	2.2	2.2	9.1	0.7
butyl isobutyrate	1.23	1.33	1.64	4.32	4.42	2.36	0.32	0.24	0.25	0.12	0.11	0.15	0.20	0.18
2-methylbutyl propanoate	6.00	6.54	8.03	13.13	9.96	5.03	0.36	0.95	0.81	0.80	0.87	1.65	0.76	0.06
3-methylbutyl 2-methylpropanoate	30.4	29.9	35.0	107.4	107.0	71.7	2.9	1.4	5.9	2.0	2.0	0.3	2.9	2.8
2-methylbutyl 2-methylpropanoate	352.3	319.0	386.0	1096.6	989.0	715.0	10.8	12.0	31.8	32.8	35.0	0.0	30.1	12.0
methyl 4-methylenehexanoate	8.0	9.5	10.2	19.3	21.8	13.8	0.37	0.55	0.96	1.60	0.25	0.30	0.63	0.35
ethyl 4-methylnonaoate	0.28	0.59	0.60	0.62	1.21	1.39	0.04	0.16	0.19	0.09	0.01	0.04	0.09	0.02
2-methylbutyl 3-methylbutanoate	6.37	7.27	9.57	23.11	31.10	29.6	1.43	0.71	0.55	2.60	1.55	0.08	2.44	1.41
2-methylbutyl 2-methylbutanoate	4.89	6.05	7.37	16.6	22.9	22.5	1.21	0.56	0.79	1.80	1.15	0.56	2.02	1.41
unidentified ethyl ester	17.6	22.9	25.4	33.6	53.0	92.5	4.1	2.4	8.7	5.0	1.4	1.9	4.0	1.8
etyhl 4-methyloctanoate	1.78	2.44	2.86	3.37	6.41	12.2	0.76	0.26	1.42	0.76	0.23	0.23	0.52	0.28
ethyl trans-4-decenoate	4.28	8.12	9.37	7.54	18.60	78.10	1.91	1.20	2.40	1.63	0.40	1.17	0.55	0.07
Sum hop esters	**481**	**467**	**555**	**1484**	**1431**	**1136**	**28.6**	**22.6**	**61.6**	**54.2**	**45.2**	**8.6**	**53.3**	**21.1**

Table 6 Monoterpene Alcohols (MTA) [µg/l]

	Mean late			Mean dry			$SD_{A\,max}$ late		$SD_{A\,max}$ dry		SD_B late		SD_B dry	
	fresh	0 °C	– 24 °C	fresh	0 °C	– 24 °C	fresh	0 °C	fresh	0 °C	fresh	0°C	fresh	0 °C
linalool	20.8	21.8	19.5	34.4	31.5	27.8	2.1	2.3	3.0	2.9	4.30	1.70	2.80	1.84
p-menth-1-en-4-ol	2.3	2.8	2.8	3.1	3.3	2.8	0.1	0.1	0.1	0.1	0.05	0.02	0.12	0.02
α-terpineol	5.4	5.6	5.9	10.4	9.6	8.4	0.3	0.2	1.1	0.1	0.36	0.14	0.79	0.14
citronellol	13.9	15.4	13.8	21.4	24.1	20.4	1.1	0.6	0.8	1.7	0.30	0.14	1.50	1.48
Sum MTA	**42.4**	**45.6**	**42.0**	**69.3**	**68.5**	**59.4**	**3.6**	**3.2**	**5.0**	**4.8**	**5.0**	**2.0**	**5.2**	**3.5**

Table 7 Oxygenated Sesquiterpenoids (OST) [µg/l]

	Mean late			Mean dry			$SD_{A\,max}$ late		$SD_{A\,max}$ dry		SD_B late		SD_B dry	
	fresh	0 °C	– 24 °C	fresh	0 °C	– 24 °C	fresh	0 °C	fresh	0 °C	fresh	0°C	fresh	0 °C
trans-nerolidol	3.01	5.93	6.37	5.11	8.21	6.27	0.56	0.55	0.95	0.33	0.26	1.24	1.01	1.46
caryophyllene oxide	41.9	28.4	35.2	38.0	38.2	14.8	15.7	3.1	20.1	2.6	4.7	1.4	4.2	0.1
humulene epoxide I	8.94	4.03	6.52	3.47	3.38	2.01	2.89	0.40	0.55	0.13	2.69	0.54	0.01	0.33
humulene epoxide II	0.37	0.42	0.45	0.54	0.83	0.59	0.15	0.09	0.11	0.12	0.13	0.09	0.04	0.06
juniper camphor	15.5	11.4	10.7	43.3	38.3	30.6	2.2	1.1	8.1	1.9	1.1	0.4	4.4	3.5
cubenol 1	3.09	2.72	2.48	5.47	5.92	3.95	0.30	0.11	0.99	0.10	0.40	0.40	0.96	0.84
sesquiterpene alcohol, unident.	12.2	15.6	14.0	28.9	37.1	22.0	1.8	0.2	7.4	2.9	0.8	1.4	0.4	3.9
γ-eudesmol	12.8	15.3	14.9	35.2	38.5	26.7	4.7	1.6	2.2	0.5	0.7	0.1	1.1	3.5
τ-cadinol	27.2	26.9	26.8	62.6	62.9	43.3	20.3	2.0	6.7	3.1	0.4	2.8	1.9	5.9
cubenol 2	4.92	3.74	3.77	8.57	7.70	5.05	1.20	0.33	2.43	0.74	0.23	0.47	0.59	1.08
β-eudesmol	4.88	5.46	4.90	13.27	14.70	10.10	1.73	0.39	0.67	1.60	0.16	0.48	0.34	1.56
α-cadinol	10.9	19.4	17.0	26.4	45.1	28.3	3.9	1.9	2.0	2.2	0.5	1.8	1.4	8.5
α-eudesmol	25.3	26.0	25.3	58.6	64.5	44.5	6.6	2.2	4.3	3.0	0.7	1.4	2.0	6.8
eudesmol - like	2.65	2.32	2.28	7.41	6.60	5.28	0.53	0.30	2.58	1.20	0.41	0.85	0.62	0.39
Sum OST	**173.7**	**167.6**	**170.7**	**336.8**	**371.9**	**243.5**	**62.6**	**14.3**	**59.1**	**20.4**	**13.2**	**13.4**	**19.0**	**37.9**

3.3 Monoterpenes (Monoterpene Hydrocarbons)

Table 3 shows that comparisons of the mean values for the two sets of samples, fresh and 0 °C, match closely with one another. The values for the analytical standard deviation hardly differ. The variation in the samples (SD_B) is lower in the 0 °C beers. However, the mean value is slightly lower for the sum of the monoterpenes in the frozen samples, indicating that deep freezing is not necessarily positive in such cases.

3.4 Sesquiterpenes (Sesquiterpene Hydrocarbons)

Table 4 shows that, in general, high values for the standard deviation must be expected for the analyses. By comparison, the calculated coefficients of variation range from 20 to over 40 %. The mean values for the beers stored at – 24 °C deviate from the other two sets of samples. There is no significant difference between “late” and “dry”. As with the monoterpenes, this suggests that freezing the samples may be problematic. The values for the sample-related standard deviation (SD_B) of the 0 °C samples are very low.

3.5 Esters Derived from Hops (Carboxylic Esters)

The mean values for the fresh samples and those stored at 0 °C correspond very closely with one another, although the data for this class of compounds also exhibit inexplicable aberrations with regard to the deep-frozen beers, as table 5 indicates. This is particularly noticeable in the dry-hopped beers (fresh = 1484 µg/l, 0 °C = 1431 µg/l, – 24 °C = 1136 µg l). This also further substantiates the presumption that deep freezing the samples results in unreliable measurements. The values for the standard deviation SD_B of the 0 °C beers are below the analytical $SD_{A\,max}$, which also underscores the reproducibility of the batches as seen in the fresh beers.

3.6 Monoterpene Alcohols

Except for the dry-hopped samples stored at – 24 °C, table 6 shows that the mean values agree very well with one another. The values for the analytical standard deviation of the beers stored at 0 °C are slightly lower than those for the fresh samples. The values for the sample-related standard deviation (SD_B) were already slightly above the $SD_{A\,max}$ in the fresh samples, which is reflected in the stored samples.

3.7 Sesquiterpene Oxides (Oxygenated Sesquiterpenoids)

Table 7 indicates that the mean values for the late-hopped beers are very close for this class of substances. However, the deep-frozen dry-hopped samples deviate considerably from the other two (fresh and 0 °C). The scatter of the data points for the multiple brews is smaller than that for the three-fold determination.

3.8 Dry hopped beers aged at – 24 °C

An interesting effect can be noticed when taking a deeper look into the mean values of hop aroma components in the dry hopped beers. In every comparison the mean of the deep frozen sample is lower than the mean of the fresh and the cold stored (0 °C) beers (table 3–7). The difference sums up to an average loss of – 27 %. This cannot be observed for the late hopped beers where no losses can be determined on average. This may be the consequence of a significantly lower concentration of the respective compounds. Obviously precipitation reactions and/or absorption cannot be excluded when freezing beers with a considerable hop addition.

Yamashita et. al. [7] reported already in 1989 about precipitations recognized when deep freezing beer. They could identify beta-glucans as the major constituents of the beer precipitates. Whether aroma substances were associated in these precipitations has not been investigated.

4 Summary

The first part of this series described the high level of reproducibility of fresh beers brewed in a 2 hl pilot brewery. In each case, the values for the standard deviation for the three late-hopped as well as the three dry-hopped beers fell within the range or below the analytical standard deviation of the three-fold determination. Particular emphasis was placed on the compounds in beer derived from hops as well as on fermentation by-products and carbonyl compounds associated with ageing. In the current study we further explored whether the mean values, the values for the analytical standard deviation and the scatter of the data points for the multiple brews were affected by storage of the beers at 0 °C and – 24 °C for 240 days. In comparison with the fresh samples, confirmation of the previously presented data was possible for the samples stored at 0 °C. It applies not only to the hop aroma compounds (monoterpenes and sesquiterpenes, their alcohols and epoxides as well as the esters derived from hops), but also to the fermentation by-products. This encompasses the mean values for the multiple brews, the analytical ranges of variance and the scatter of the data points within the multiple batches. However, Strecker aldehydes do not follow this pattern: an increase, even after moderate ageing when beer is stored at 0 °C for 240 days, is observed. Collecting beer samples over a longer period and performing all of the analyses at once would therefore not seem to be a problem.

Cold storage of hoppy beers over 240 days at 0 °C has very little effect on the hop aroma compounds examined in these trials. Deep-freezing the samples resulted in a reduction of most of the hop aroma compounds in the dry-hopped beers compared to the fresh beers stored at 0 °C. There is no obvious explanation for this phenomenon but it indicates that freezing is not an option for preserving beer samples for a later analysis of hop aroma compounds as precipitation and/or absorption cannot be excluded.

A follow-up report will be issued on the transfer rates of hop aroma compounds in beer and on ageing trials conducted at 0 °C, 4 °C, 20 °C and 30 °C for 470 days.

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