M. Geserer, F. Peifer, M. Krottenthaler, K. Schmid and S. Cocuzza

How the amount and timing of dry-hopping affects beer turbidity

The aim of this study was to investigate the effects on beer turbidity after dry-hopping. Firstly, depending on the quantity of hop pellets added and secondly, when varying the dosing point while maintaining a constant hopping rate. Selected chemical and physical properties of the finished beer were also investigated for both scenarios (quantity and time of dry-hopping). Both the “trial quantity” (TQ) and “trial timing” (TT) resulted in significant effects with regards to the increase in turbidity in the dry-hopped beers. Gradually increasing dry-hop dosing rates in TQ correlated well with the greater turbidity according to both methods used, MEBAK and OD600. All dry-hopped beers in TT showed a significant increase in turbidity compared to the base beer. This haze was further intensified with a very late dry-hop dosage at the end of maturation. The turbidity was, along with other selected variables, also analysed after the beer had been stored for 6 months at 20 °C, “trial storage” (TS). Both, the supernatant formed after storage and the homogenised beers were investigated. The results of the stored and homogenised samples showed highly comparable and, in some cases identical results compared to analyses of freshly bottled beers of TQ and TT. The effect of the dry-hopping variations of TQ and TT could still be demonstrated analytically in the supernatants and visually in the settled beers. Pictures were taken to visualise the behaviour of the various dry-hopped beers to link the results to the visual expectations that consumers have of commercially available beers.

Descriptors: dry-hopping, turbidity, 90 °/ 25 ° measurement, OD600, bitter substances, polyphenols, pH value, real extract and alcohol content, hop creep

1 Introduction

Dry-hopped beer styles have become an integral part of today‘s beer market and their production methods vary in many ways: selection of hop varieties, quantity of hops, type of hop product, time of addition and dosing technique, to name just a few ways of adjusting the process using hops. The main reason to add hops on the cold side of beer production is to impart an intense and unique hop aroma, which is a typical and important attribute of such beer styles. In addition, a wide variety of malts and other cereals are used in dry-hopped beer styles to influence and define the characteristic flavour and the physical and chemical attributes of the beer. In most cases these types of beers undergo hardly any filtering. Similarly to other beer styles such as wheat beer, various Belgian beer styles and unfiltered craft or organic beers, consumers expect and desire a certain haziness [13]. Therefore, a hazy appearance is an important criterion for consumers, not only when looking at the bottled beer in the supermarket but also after pouring it into a glass. It is not uncommon for haze to reduce or become inhomogeneous during storage. In some cases, sedimentation or an unappealing flaky or coarse flocculation may even occur [6]. The New England IPA (NEIPA) is at the end of the range when it comes to hazy beers [14]. The very high load of haze-active proteins (primarily introduced by high-protein adjuncts such as oat flakes and/or wheat) can even act as a carrier for non-polar hop-derived bitter and aroma substances, resulting in extremely high concentrations scarcely reached in any other type of beer [3].

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Beer turbidity is generally categorised as biological or non-biological (colloidal) haze, with the latter further divided into permanent and chill haze [22]. Typically, turbidity or haze is measured by detecting the light scattered by particles in the sample at two different angles. Particles below 1 µm can be detected at an angle of 90 °, and particles above 1 µm at an angle of about 25 ° forward scattering. The turbidity is measured with a turbidity meter using a series of formazin dilutions as standards [15].

Biological haze is the result of residual yeast cells in unfiltered beers but can also come from microbial contamination in any kind of filtered or unfiltered beer [19]. The non-biological turbidity of unfiltered and non-stabilised beer is primarily influenced by the beer’s protein content and the distribution of the particle sizes in these haze-active proteins [8]. They originate from various brewing raw materials and can interact with haze-active polyphenols [2] e.g. proanthocyanidins (oligomeric flavonoids), dimers, and tetramers of catechin, epicatechin, and gallocatechin [22].

In the past, the basic rule for introducing polyphenols was that around two thirds came from the malt and one third from the hops. As it has become more popular to dry-hop with extremely high volumes of hops, the ratio has shifted towards more influence from hops in general. As a result, the interactions between protein fractions and polyphenols from all raw materials, and especially from hops, are more pronounced during and after dry-hopping [11]. Other notable factors that can influence the turbidity of beer include the effects of polypeptides, polysaccharides, mineral substances, and oxygen [19].

Dry-hopping itself strongly influences the overall beer matrix and non-volatile components also undergo major changes once hops are added to the cold side of beer production. Studies have shown significant changes in the beer's bitter acid composition, pH value and foam stability [5, 11]. Changes also occur in original gravity and real extract as well as alcohol formation after dry-hopping, due to the hop creep effect, which has been subject to in-depth investigation [23, 24]. With regards to the increasing polyphenol content, it remains unclear whether there's a linear increase caused by the quantity of hop pellets added, or if solubility might somehow be limited due to direct interactions with proteins, followed by separation with the cold trub. In a pilot-scale study, *Parkin* et al. observed that the water-soluble polyphenols from hops were found in higher concentrations in dry-hopped beer depending on the quantity added and a longer extraction. Higher polyphenol concentrations also increased the corresponding analytical IBU values (and to a minor extent the sensory perception of bitterness). The additional study of 15 commercial dry-hopped beers showed both higher and lower polyphenol concentrations after dry-hopping [21]. *Cocuzza* et al. observed an averaged polyphenol increase of 4.6 mg/l per 100 g/hl hop pellets added during dry-hopping, with a maximum increase of 98 mg/l for 1500 g/hl dosed. Considering the base beer, this corresponds to an increase of 25 % respectively [4] and follows a similar observation by *Oladokun* et al. as well as Parkin et al. (15 – 30 %) [20, 21]. Turbidity was also analysed, but no clear conclusion could be made. This was due to the analytical variation being too high to draw conclusions about haze formation and a possible link to the polyphenols introduced [4].

Janish refers to a study where a polyphenolic extract from spent hops was used for dry-hopping, resulting in more haze being formed. High transfer rates of polyphenols from whirlpool and dry-hopping are also described. Rates of up to 35 % are possible for dry-hopping and the high input of polyphenols ultimately leads to an irreversible and permanent haze. As far as the influence of pH is concerned, it is concluded that a low pH has less influence on haze formation, which is the case with dry-hopping after fermentation [10].

The study described here was conducted to further understand the interaction between hops and traditionally dry-hopped beer using hop pellets. Two approaches were investigated in detail, analysing the freshly brewed beer and after storage of 6 months:

- varying the quantity of hop pellets added at the same timing;
- varying the timing of a constant dosage followed by constant contact time.

2 Materials and Methods

2.1 Hop pellets

US Cascade was chosen for dry-hopping as it is typical of the varieties often used to produce dry-hopped beers. Table 1 provides an analytical characterisation of the hop pellets of crop 2021 used in this investigation.

Table 1 Characterisation of the used hop pellets

	Method [9]	**Type 90 pellets**
Lead conductance value	EBC 7.5	6.0 %
Alpha acids	EBC 7.7*	5.4 %
Humulinones	EBC 7.7*	0.5 %
Polyphenols	EBC 7.14	3.5 %

* the most recent international standards were used for the calibration

2.2 Non-dry-hopped base beer recipe

The base beer was produced according to Hopsteiner's pale ale recipe. The original gravity of the wort was 14 °Plato and was produced with a grain bill consisting of 39 % pilsner malt, 30 % Vienna malt, 15 % pale caramalt, 15 % wheat malt and 1 % acidulated malt. Mashing was carried out according to a Hochkurz (from German, meaning high and short) mash program with a mash-in temperature of 65 °C. This temperature was maintained for 30 minutes followed by heating up to 72 °C and a subsequent rest of 30 minutes. After a positive iodine normality test, the mash was heated up to 78 °C and transferred to the lautering tun. After lautering the wort was boiled for 70 minutes with two hop additions. The first addition took place at the beginning of the boil at which time 80 % of the total alpha acids was added, followed by another 20 % of the alpha acids after 60 minutes. The hop additions were calculated to achieve 14 IBU in the base beer prior to dry-hopping. For each addition, US Cascade type 90 pellets from the same batch were used (Table 1). Wort boiling was followed by a whirlpool rest of 20 minutes and cooling to an initial fermentation temperature of 20 °C. Fermentation was conducted at atmospheric pressure with the Fermentis S04 strain. This base beer was used in all of the dry-hopping trials described below.

2.3 Dry-hopping technique (static) & beer production

First, two brews of the single hop base beer were produced and the yeast was pitched as described in 2.2. These two young beers were the basis for the subsequent dry-hopping experiments. In trial quantity (TQ) the additions were conducted in the middle of maturation on day 8 using hop rates of 0, 250, 500, 750, 1000 and 1500 g/hl. For the timing trial (TT) a dry-hopping rate of 750 g/hl was chosen for all additions, which were conducted on days 1, 2, 3, 7 and 10 after pitching the yeast.

Each hop addition was performed using identical settings for the dry-hop. The pellets were dissolved in the base beer using a magnetic stirrer in a beaker that was continually flushed with CO_2 to avoid oxygen intake. The resulting hop slurry was added to NC

Table 2 Overview of applied beer analyses

Methods	
MEBAK online; B-590.10.181 [17]	Alcohol [% vol.], Real extract [% w/w]
MEBAK online; B-590.00.040 [16]	pH value
MEBAK online; B-420.01.272 [15]	Turbidity [EBC]
Optical Density at 600 nm	Extinction
EBC 9.8 [9]	Bittering units [IBU]
EBC 9.11 [9]	Polyphenols [mg/l]
EBC 9.47 modified (HPLC)* [9]	Alpha acids, Iso-alpha acids, humulinones [mg/l]

* the most recent international standards were used for calibration

kegs on a scale via the manhole while flushing the keg from below with CO_2 through the outlet pipe. The manhole was closed and the keg was put on a scale to be subsequently filled to 15 kg of beer, including the beer of the hop slurry but without the weight of the corresponding pellet quantity. This enabled homogenous distribution of the dry-hop dosage and rapid onset of any extraction and metabolising effects. The beer was then kept at a temperature of 20 °C and the kegs were inverted twice during the first two days after the dry-hop application to mix the contents and improve extraction of the hop components into the beer. All beers in TQ were cooled down 7 days after the dry-hop to enable hop-derived metabolization and reaction. The beers in TT were cooled down 7 days after completion of the main fermentation regardless of their respective timing for the dry-hop. In both trials the beers had 11 days of contact time with the pellets. The kegs were equipped with shortened extractor tubes, so that after dry-hopping, the beer could be transferred to 10-litre NC kegs without the yeast sediment and the majority of hop particles and cold trub respectively. All of the trial beers were stored at 5 °C for two weeks and carbonated to a standard concentration of 5.2 g/l of CO_2. The unfiltered beers were then bottled in flint glass bottles for turbidity analysis and visual evaluation as well as regular brown glass bottles for chemical and physical analyses.

Table 3 All mean values for trial quantity (TQ) fresh and aged (6 months, 20 °C) where applied

		Dry hop dosage Cascade P90 [g/hl]	0	250	500	750	1000	1250	1500
Turbidity analyses	90° [EBC]	fresh	53.7 ± 0.9	84.5 ± 2.4	120.3 ± 9.9	174.3 ± 2.5	>200	>200	>200
		aged homogenised	79.7 ± 18.5	74.3 ± 8.4	125.3 ± 4.0	179.3 ± 4.0	>200	>200	>200
		aged supernatant	3.1 ± 0.2	7.3 ± 0.2	13.6 ± 0.3	20.3 ± 0.6	27.0 ± 0.5	35.6 ± 0.9	42.9 ± 0.9
	25° [EBC]	fresh	75.3 ± 1.3	99.2 ± 3.6	137.4 ± 10.1	198.3 ± 3.5	>200	>200	>200
		aged homogenised	91.7 ± 1.3	79.4 ± 8.4	131.3 ± 5.5	182.3 ± 4.7	>200	>200	>200
		aged supernatant	7.5 ± 0.4	6.8 ± 0.1	11.3 ± 0.5	15.0 ± 1.2	18.4 ± 0.2	23.7 ± 0.9	28.6 ± 0
	OD600	fresh	0.37 ± 0.007	0.49 ± 0.008	0.62 ± 0.034	0.84 ± 0.004	1.03 ± 0.026	1.22 ± 0.044	1.35 ± 0.038
		aged homogenised	0.54 ± 0.058	0.59 ± 0.010	0.77 ± 0.029	0.98 ± 0.037	1.13 ± 0.036	1.38 ± 0.082	1.47 ± 0.043
		aged supernatant	0.09 ± 0.006	0.09 ± 0.006	0.10 ± 0.005	0.14 ± 0.009	0.17 ± 0.002	0.21 ± 0.003	0.28 ± 0.014
Bitter analyses	IBU [EBC]	fresh	14.8 ± 0.10	28.1 ± 0.15	36.2 ± 0.46	44.1 ± 0.56	51.1 ± 0.15	58.8 ± 0.42	62.0 ± 1.37
		aged	12.3 ± 0.49	23.6 ± 0.06	29.6 ± 0.40	36.6 ± 1.30	44.2 ± 0.10	52.3 ± 0.64	51.8 ± 0.67
	Bitter acids [mg/l]	iso-α-acids fresh	10.0 ± 0.00	9.0 ± 0.16	8.6 ± 0.21	8.1 ± 0.00	7.7 ± 0.12	7.7 ± 0.00	7.5 ± 0.12
		iso-α-acids aged	8.3 ± 0.88	8.6 ± 0.12	8.2 ± 0.06	7.8 ± 0.06	7.6 ± 0.12	7.6 ± 0.06	7.4 ± 0.15
		α-acids fresh	0.9 ± 0.12	8.8 ± 0.36	10.7 ± 0.30	14.3 ± 0.35	17.0 ± 1.86	17.6 ± 0.91	22.4 ± 1.62
		α-acids aged	0.3 ± 0.06	4.4 ± 0.57	5.4 ± 0.17	7.6 ± 1.07	10.1 ± 0.40	12.2 ± 0.21	13.4 ± 0.95
		Humulinones fresh	1.4 ± 0.06	8.8 ± 0.25	15.5 ± 0.21	23.7 ± 0.17	30.0 ± 0.20	36.0 ± 0.15	40.2 ± 2.02
		Humulinones aged	0.8 ± 0.17	5.1 ± 0.31	9.5 ± 0.10	14.7 ± 0.87	19.8 ± 0.27	24.7 ± 0.50	30.6 ± 0.99
		polyphenols [mg/l]	191 ± 7.4	203 ± 23.5	235 ± 25.5	284 ± 40.5	352 ± 15.1	342 ± 9.1	356 ± 12.8
Standard beer analyses		alcohol [vol.-%]	5.64 ± 0.020	6.13 ± 0.006	6.30 ± 0.027	6.37 ± 0.010	6.45 ± 0.020	6.44 ± 0.029	6.49 ± 0.023
		real extract [%w/w]	5.68 ± 0.049	4.99 ± 0.012	4.76 ± 0.000	4.66 ± 0.006	4.64 ± 0.010	4.66 ± 0.025	4.70 ± 0.032
		pH	4.35 ± 0.021	4.35 ± 0.006	4.43 ± 0.006	4.54 ± 0.012	4.64 ± 0.006	4.70 ± 0.006	4.75 ± 0.006

2.4 Real-time storage

Each individual trial included real-time storage for 6 months at 20 °C. Following this period, each beer was analysed with regards to turbidity (MEBAK as well as OD600) and HPLC analysis of bitter components was performed. Beers filled in flint bottles for further visual inspection were stored away from light. The supernatant that formed above the sediment in the beer samples was examined separately. Then the sample was subjected to standardised shaking, and the homogenised beers were analysed. Photos were also taken to visualise the beers' haze over time. The results of the storage trials are shown in the sections named trial storage (TS).

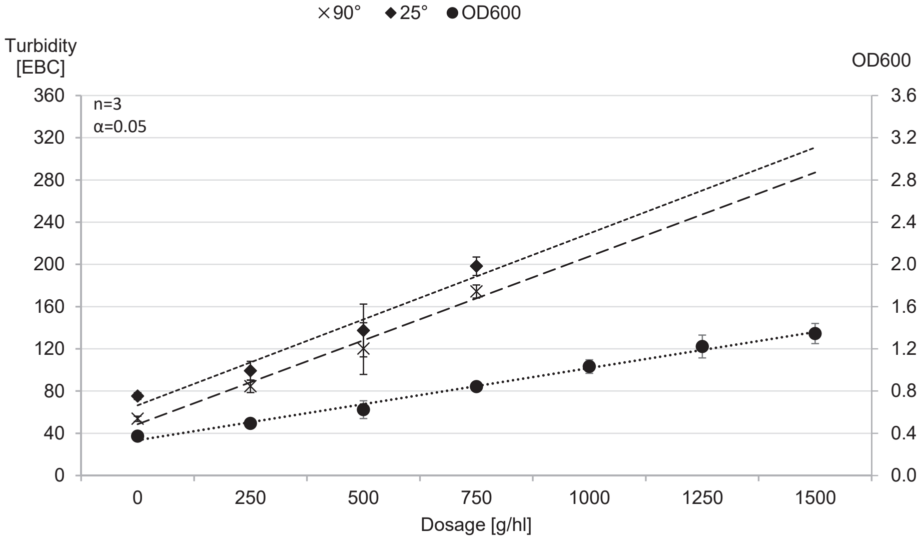

Fig. 1 TQ, turbidity 90/25°, OD600, fresh, confidence intervals superimposed on the graph

2.5 Beer analyses

Table 2 shows the methods applied to analyse the finished beers. All initial analyses were performed within two weeks of bottling or after the storage trial of 6 months.

Turbidity 90 °/25 °: In this set-up, clear 0.5 l NRW bottles were chosen for use as cuvettes in the Sigrist Labscat2. This enabled all the turbidity analyses to be performed from the same bottle or beer sample to avoid measurement fluctuations. However, measurements were limited to 200 EBC units for this device.

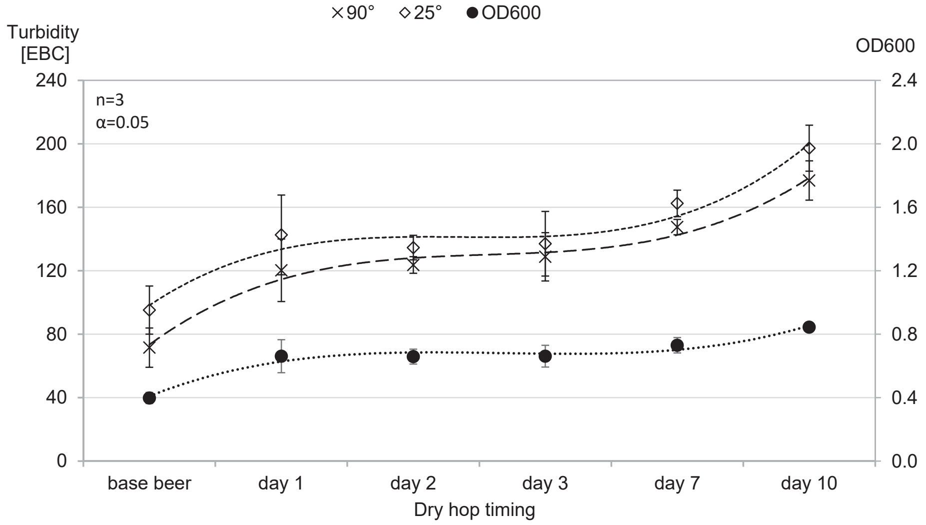

Fig. 2 TT, Turbidity 90/25°, OD600, fresh, confidence intervals superimposed on the graph

OD600 was introduced to give a complete overview of the turbidity of all dry-hopped samples. OD600 is typically used to estimate the concentration of bacteria in a liquid. Each sample is measured against distilled water in a spectrophotometer at 600 nm and 20 °C. The extinction provided by the spectrophometre is made up of the absortion and the scattering of light within the sample.

3 Results and Discussion

Each trial and dry-hopping dosage was performed in triplicate, while each analysis was performed in duplicate.

3.1 Turbidity EBC 90 °/ 25 ° and OD600

3.1.1 Trial quantity (TQ)

Average turbidity values for TQ are displayed in table 3. The initial turbidity in the base beer was 53.7/ 75.6 EBC units (90 °/ 25 °). Increasing hop dosages correlated with rising turbidity values and deltas of 13.9/ 12.8 EBC units (90 °/ 25 °) for each 100 g pellets per hl added. A dry-hopping dosage of 750 g/hl reached values of 174.3/ 198.3 EBC (90 °/ 25 °). Turbidity analyses of beers that were dry-hopped with 1000 g/hl and more exceeded 200 EBC units and could not be measured within this specific setup. Figure 1 presents the mean turbidity results at a 95 % confidence interval.

The absorbance at 600 nm measured using a spectral photometer showed an initial value of 0.37 for the base beer and an increase of 0.07 for each 100 g/hl pellets dosed. The highest value of 1.35 could be observed for the beer dry-hopped with 1500 g/hl, the highest applied dosage (Fig. 1, Table 3).

3.1.2 Trial timing (TT)

Mean turbidity values for TT are displayed in table 4. The results are summarised in figure 2.

Table 4 **All mean values for trial timing (TT) fresh and aged (6 months, 20 °C) where applied**

		Dry hop dosage Cascade P90 timing	Base beer	day 1	day 2	day 3	day 7	day 10
Turbidity analyses	90° [EBC]	fresh	71.5 ± 4.9	120.3 ± 7.9	123.6 ± 1.9	128.8 ± 6.1	147.5 ± 1.9	176.9 ± 4.9
		aged homogenised	61.7 ± 11.1	134.3 ± 8.4	131.8 ± 8.3	146.0 ± 9.0	163.7 ± 3.2	184.3 ± 2.1
		aged supernatant	6.3 ± 0.5	4.0 ± 0.5	18.1 ± 0.7	20.2 ± 0.7	19.5 ± 0.5	21.9 ± 0.9
	25° [EBC]	fresh	95.2 ± 6.1	142.6 ± 10.1	134.6 ± 2.8	137.0 ± 8.2	162.4 ± 3.4	197.3 ± 5.8
		aged homogenised	73.5 ± 9.2	148.3 ± 7.5	138.5 ± 8.3	153.0 ± 10.5	168.7 ± 3.2	186.3 ± 2.1
		aged supernatant	8.1 ± 0.3	6.8 ± 0.6	15.0 ± 0.8	14.6 ± 0.7	12.0 ± 0.3	13.7 ± 1.6
	OD600	fresh	0.39 ± 0.014	0.66 ± 0.042	0.66 ± 0.019	0.66 ± 0.028	0.73 ± 0.019	0.85 ± 0.011
		aged homogenised	0.51 ± 0.017	0.80 ± 0.039	0.79 ± 0.029	0.81 ± 0.031	0.93 ± 0.025	0.91 ± 0.123
		aged supernatant	0.08 ± 0.014	0.05 ± 0.003	0.11 ± 0.006	0.14 ± 0.001	0.15 ± 0.008	0.14 ± 0.013
Bitter analyses	IBU [EBC]	fresh	14.8 ± 0.10	39.5 ± 0.15	38.9 ± 0.21	40.7 ± 0.49	42.5 ± 0.31	44.1 ± 0.46
		aged	13.2 ± 0.15	32.1 ± 0.10	–	–	–	38.7 ± 0.31
	Bitter acids [mg/l]	Iso-α-acids fresh	11.1 ± 0.12	8.2 ± 0.12	7.8 ± 0.15	8.2 ± 0.12	9.1 ± 0.20	9.4 ± 0.06
		Iso-α-acids aged	9.3 ± 0.25	8.2 ± 0.06	–	–	–	9.2 ± 0.15
		α-acids fresh	0.9 ± 0.12	12.6 ± 0.40	11.1 ± 0.81	11.9 ± 0.32	11.7 ± 0.52	14.7 ± 0.35
		α-acids aged	0.5 ± 0.15	6.9 ± 0.12	–	–	–	9.5 ± 0.40
		Humulinones fresh	1.4 ± 0.00	21.4 ± 0.81	21.9 ± 0.40	22.4 ± 0.42	24.1 ± 0.17	23.6 ± 0.46
		Humulinones aged	0.8 ± 0.10	12.2 ± 0.15	–	–	–	15.7 ± 0.10
		Polyphenols [mg/l]	179 ± 6.0	256 ± 7.0	247 ± 6.2	258 ± 7.6	267 ± 11.0	277 ± 11.7
Standard beer analyses		Alcohol [vol.-%]	5.74 ± 0.002	6.40 ± 0.027	5.75 ± 0.329	6.08 ± 0.035	6.09 ± 0.147	5.73 ± 0.010
		Real extract [%w/w]	5.17 ± 0.006	4.25 ± 0.006	4.52 ± 0.067	4.38 ± 0.017	4.51 ± 0.186	5.41 ± 0.012
		pH	4.38 ± 0.022	4.54 ± 0.010	4.40 ± 0.010	4.43 ± 0.010	4.55 ± 0.000	4.63 ± 0.006

Comparing the turbidity results for the set pellet addition (750 g/hl) at different timings with the base beer, dry-hopping leads to a significant impact on beer turbidity at any stage of fermentation as well as at maturation. The lowest turbidity was measured in the base 71.5/ 95.2 EBC (90 °/ 25 °). The highest turbidity was observed for the dry-hop timing on day 10 at 176.9/ 197.3 EBC (90 °/ 25 °), resulting in a maximum delta of 105.4/ 102.1 EBC (90 °/ 25 °). The impact was lower for dosages during active fermentation/ mid maturation (days 1 to 7) compared to the late dry-hopping on day 10. A consistent average increase of 58.5/ 48.9 EBC (90 °/ 25 °) was observed from days 1 to 7, with no significant difference between the individual timings of dry-hopping. The beers ranged from 120.3 to 147.5 EBC units for 90 ° and 130.5 to 162.4 EBC units for 25 °. A trend can be seen for increased turbidity starting on day 7 (Fig. 2). This indicates that a later timing for the dry-hopping either enhances the processes responsible for the formation of haze or inhibits processes that reduce existing turbidity. A possible explanation for this could be the decreased fermentation activity when hops are added much later in the process.

The pattern of the values for 25 ° and 90 ° were highly comparable (Fig. 2) ,which shows a size-independent behaviour of the turbidity-forming particles.

The initial value of 0.39 in TT is close to the initial value of TQ, which indicates that there are practically no differences to the base beer, the setting or the handling. After dry hopping with pellets (750 g/hl) at various times, the OD600 reached a very compareable level around 0.7 to the corresponding trial with TQ (750 g/hl on day 8). The maximum delta could be observed on day 10 at + 0.45 (Fig. 2, Table 4).

3.1.3 Trial storage of TQ-samples

The observations for the analysis results of the homogenised samples of all dry-hopped beers after 6 months of warm storage at 20 °C were very similar, or in some cases identical, to those of the fresh samples (Fig. 3). The mean results are shown in table 3. The non-dry-hopped base beer did not show a significant difference to the beer dry-hopped at a rate of 250 g/hl.

OD600 showed comparable behaviour to the turbidity at 90 °/ 25 ° correlating with both values (Fig. 4).

For the analyses of the supernatants of the settled beers, it was possible to observe a linear increase of turbidity at 90 ° and 25 °, with the biggest respective increase for 1500 g/hl (90 °: 42.9 EBC; 25 °: 28.6 EBC) (Table 3).

The OD600 results for the supernatants show a linear increase in turbidity for all beers dry-hopped from 750 to 1500 g/hl compared to the base beer (Table 3).

The consistency of analysis results of 90 °/ 25 ° of the aged samples compared to the fresh ones revealed no significant difference and indicates that the particle sizes and their distribution within the formed haze remain unchanged throughout the warm storage trial.

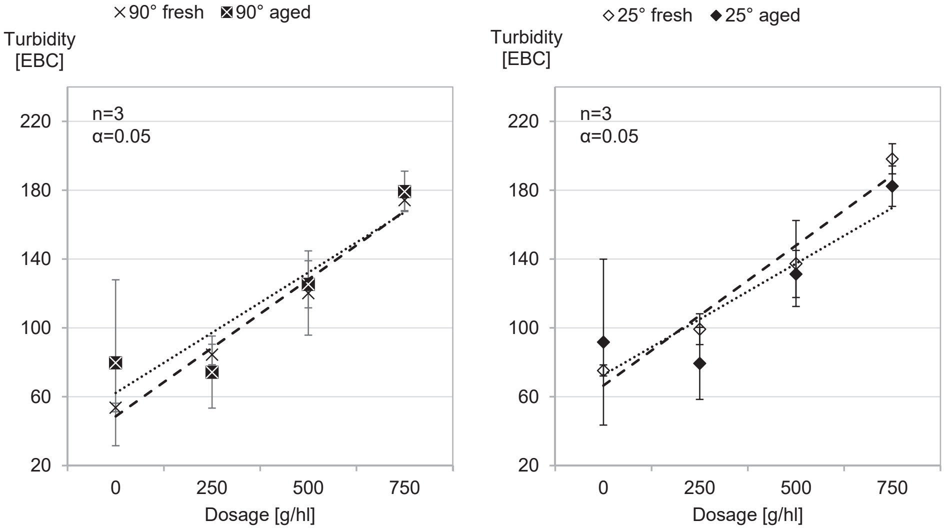

Fig. 3 **TQ, homogenised, turbidity 90(l)/25°(r), fresh vs. aged, confidence intervals superimposed on the graphs**

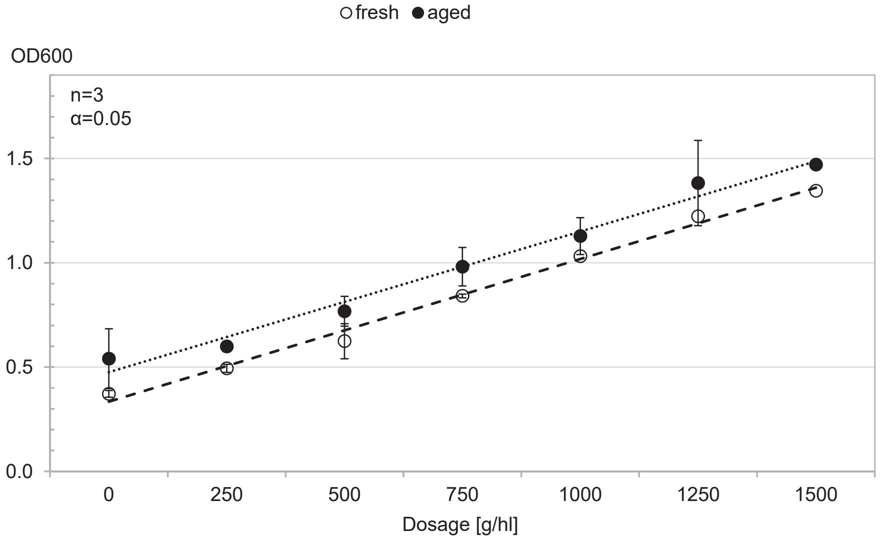

Fig. 4 **TQ, homogenised, OD600, fresh and aged, confidence intervals superimposed on the graph**

The appearance of the bottled beer of all single trials of TQ (Fig. 5) confirms the observation in the analysis results. An increase in haze can be seen in the settled beers after 6 months of warm storage away from light. For the beers dry-hopped at a volume of 500 g/hl and above, a stable haze has developed, which is obviously hazier at higher hop dosages. The samples of 750 to 1500 g/hl show increased haze in the settled beers.

Fig. 5 **TQ; visual appearance after 6 months of warm storage away from light; left to right: 0, 250, 500, 750, 1000, 1250, 1500 g pellets per hl**

3.1.4 Trial storage of TT-samples

The homogenised samples after 6 months of warm storage at 20 °C generally showed no significant difference to the fresh samples (Fig. 6). OD600 confirmed these findings (Fig. 7). The mean values are shown in table 4.

When investigating the supernatant analyses, all beers dry-hopped after day 1 of fermentation had a significantly increased 90 °/ 25 ° turbidity compared to day 1 with an average increase of 5.7/ 13.6 EBC units (90 °/ 25 °) (Table 4).

The results for OD600 generally show an increase for all beers dry-hopped from day 2. These results are not significant due to

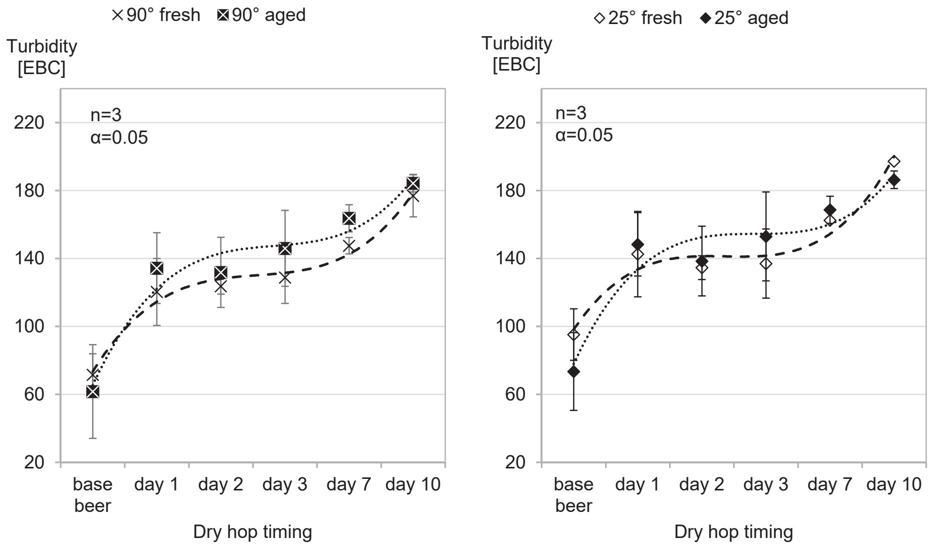

Fig. 6 TT, homogenised, turbidity 90(l)/25°(r), fresh and aged, confidence intervals superimposed on the graphs

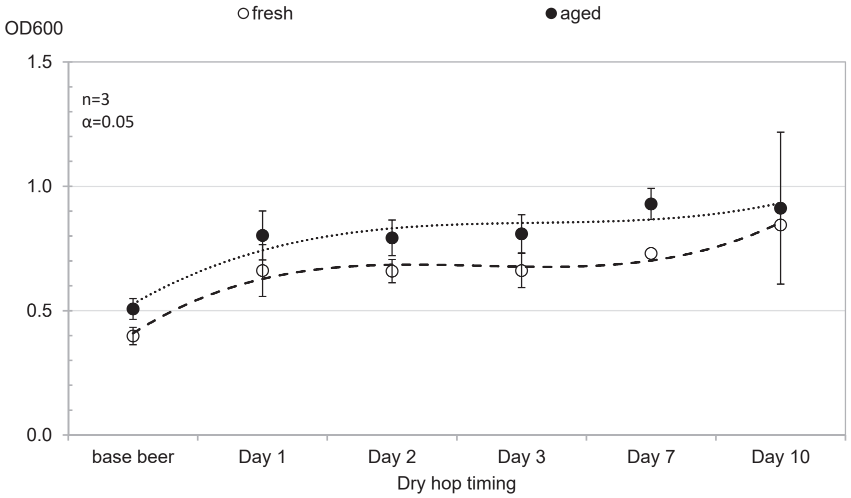

Fig. 7 TT, homogenised, OD600, fresh and aged, confidence intervals superimposed on the graph

Fig. 8 TT; visual appearance after 6 months of light-protected warm storage; left to right: base beer, day 1, day 2, day 3, day 7, day 10

big confidence intervals and suggest that OD600 is more accurate for samples with more intense turbidity (Table 4).

According to the above findings, no change in the quality of the haze occurred during storage. No clear differences were observed in the ratio of 90 ° and 25 ° turbidity, which indicates stable particle sizes and distribution and typical settling behaviour that is dependent upon the particle-size.

Visual evaluation of the single trials of TT (Fig. 8) after 6 months of warm storage indicates increased turbidity for dry-hop dosages that are added later in the production process.

3.2 Other observations

3.2.1 Polyphenols

The mean results of the polyphenol analyses are depicted in table 3 for TQ and in table 4 for TT.

TQ: The initial polyphenol content of the base beer was 192 mg/l, reaching 356 mg/l for the beer dry-hopped at a rate of 1500 g/hl. This corresponds to a maximum increase of 86 %, and therefore a higher increase compared to other studies [5, 20]. However, these studies had higher initial polyphenol concentrations in their base beers which may have limited any further increase after dry-hopping.

While the dosage rates from 250 to 750 g/hl showed no significant difference of polyphenols compared to the base beer, the dosing rates from 1000 to 1500 g/hl displayed a significant increase compared to dosages ranging from 0 to 500 g/hl. Again, the analysis results for 1000 to 1500 g/hl were all at the same level. A linear correlation for the increase of polyphenols with incremental hop dosing rates can be observed in the dose response curve, which is in line with the results of Parkin et al. [21].

TT: The initial polyphenol content of the base beer was 179 mg/l, reaching 278 mg/l for dosage on day 10. This increase of almost 55 % in the total polyphenol content corresponds very well to Oladokun et al. [20] who found a 49 % increase after 3 days. For Oladokun, this faster increase was observed under a dynamic dry-hopping procedure, which usually results in the faster extraction of hop-derived components.

All dosage timings from day 2 onwards showed a significant increase of polyphenols when compared with the base beer.

3.2.2 Bitterness units and bittering substances

The mean results for the bitter substance analyses are given in table 3 for TQ and table 4 for TT.

TQ: Compared to the initial IBU value of 14.8 in the base beer, each dry-hopped beer had correlating increased bittering units after dry-hopping, rising to a delta of 47.8 IBU at the highest dosing rate of 1500 g/hl. These observations confirmed the findings of other studies [4, 14, 18, 21].

The other bitter components varied with increasing dosing rates. The iso-alpha acid decrease correlated well with the increased pellet dosage up to 1000 g/hl. Pellet additions above 1000 g/hl resulted in iso-alpha acid contents on a stagnating level.

Concentrations of alpha acids and humulinones increased when greater hop volumes were added to the maturing beer. A significant increase in alpha acids was detected after dry-hopping. For lower dosing rates from 250 to 750 g/hl, a significant increase was observed in each case. No significant increase could be determined at higher dosages from 1000 to 1500 g/hl. At the highest dosing rate of 1500 g/hl, the maximum increase detected was + 20.8 mg/l. When measuring the concentrations of humulinones, a total increase of 38.8 mg/l was detected, a concentration 27 times higher than in the base beer. Due to the hydrophilic properties of humulinones as reported by *Maye* et al. [14], high solubility was to be expected. The sensorial bittering impact of humulinones is considered to be 66 % of that of iso-alpha acids [1], which emphasises their relevance for dry-hopped beers. All beers showed an average increase of 2.8 mg/l humulinones per 100 g/hl pellets added.

TT: The timing of the dry-hop application did not affect the IBUs of the beers at all, but each dry-hopped beer had a significantly higher IBU value compared to the base beer. On the contrary, all dry-hopped beers had significantly lower iso-alpha concentrations than the base beer. The later the beer was dry-hopped, the lower the loss of the iso-alpha acids, with the exception of day 1. No significant difference was observed between the different dosing timings for the alpha acids and the humulinones.

TS: The real-time aging for six months at 20 °C had a significant impact on the matrix of all beers, causing a decrease of IBUs, alpha acids and humulinones. The iso-alpha acids in the beers were statistically unaffected. (Tables 3, 4).

3.2.3 pH value

Mean analysis results for pH are shown in table 3 for TQ and table 4 for TT.

TQ: As expected, the maximum increase of the pH value of 0.40 was observed when comparing the base beer to the beer with the highest dry-hopping dosage rate. A significant increase in the pH value was observed from 500 to 1500 g/hl, a pH of 0.02 per each additional 100 g/hl pellets. When comparing the pH of all dry-hopped beers, there was an average increase of 0.02 per 100 g/hl with a linear correlation to the dosing rates. Our results are below what could be expected when looking at the most recent papers [5, 12, 14], which indicated an average increase of 0.03 to 0.036 per100 g/hl pellets. Based on these observations, we conclude that the pH value increases in any case after a beer is dry hopped. Depending on the impact to be expected from a dry-hop dosage, it might be useful to consider adjusting the wort pH to counteract the pH increase.

TT: The beer dry-hopped on day 1 shows a significant pH increase of 0.17. Deltas of 0.07, 0.18 and 0.26 were measured for days 3, 7 and 10 respectively, while the pH on day 2 was not significantly affected. Day 7 was the middle of maturation, which ended on day 10. The beer was subsequently cooled down to 5 °C. This indicates that the later that dry-hopping takes place, the greater the expected pH increase due to lower yeast activity and less influence of the fermentation process on the hop compounds that influence the pH. Day 1 is an exception to the rule.

3.2.4 Real extract and alcohol content (by volume)

The mean analysis results for alcohol and real extract are shown in table 3 for TQ and table 4 for TT.

TQ: Overall, the real extract decreased by 0.98 % w/w in conjunction with the highest hop dosage and by 0.69 % w/w with the lowest dosage of 250 g/hl, although all beers dry-hopped between 500 and 1500 g/hl showed no significant difference from each other. This indicates that the hop-derived amylases saccharify the non-fermentable extract of the beer and the extract introduced by the pellets is mostly negligible. However, Cocuzza et al. [5] and *Lafontaine* et al. [12] observed a linear increase in the real extract. In their trial setups, dry-hopping additions were performed in the finished beer, meaning that the extract was saccharified but not metabolised. This was contrary to this studies set up in which dry-hopping was performed in the presence of active yeast. In contrast, alcohol increased in correlation to the extract decrease, providing a good example of the hop creep effect [23, 24].

TT: Real extract decreased and alcohol increased for all dry-hop timings except for day 10, which gives a good indication of hop creep potential [23, 24] The real extract of this beer increased by + 0.2 % w/w compared to the base beer. However, cooling down to 5 °C on day 11 limited the time for the yeast to metabolise the available extract, resulting in no change to the alcohol content. In commercial brewing, this might be problematic on many levels, at least for non-pasteurised or non-sterile filtered beers. Storing at warm temperatures bears the risk of refermentation occurring in the container. Dry-hopping during active fermentation helps to keep subsequent issues in check, such as bombage of bottles due to overcarbonation.

4 Conclusion

Gradually increasing dosing rates for dry-hopping as well as varying the timings of adding type 90 pellets during fermentation and maturation were investigated with regard to their impact on the turbidity of the finished beer. The resulting changes to the non-volatile characteristics of the beer matrix were also examined. Each beer was additionally stored in real time for 6 months at 20 °C and subsequently evaluated by analysis and visual examination.

The study revealed several effects of dry-hopping with pellets on the turbidity of the produced beers. Incremental dosing rates correlate well with higher turbidity. The analysis results show average increases of 13.9 EBC units for 90 ° turbidity and 12.8 EBC units for 25 ° turbidity for each 100 g pellets per hl added. The OD600 absorbance also increases by 0.07 per 100 g/hl, confirming this influence.

Dry-hopping at all the examined dosing timings during fermentation and maturation has an impact on the turbidity. This effect is greater when dry-hopping occurs later in the process, especially once fermentation is complete. Beers dry-hopped on or after day 2 of fermentation show significant turbidity increases compared to the base beer.

Visual appearance clearly changes over time, if bottles are not moved. The supernatant analysis performed after the beers had been stored for 6 months at 20 °C, indicates a trend for increasing turbidity with increasing dry-hopping volumes as well as with additions later in the process. This trend can also be observed in the photos of the flint bottles after long-term storage in a dark place. After homogenizing the formed sediments no statistical difference between the turbidity of fresh and stored samples can be observed. This indicates that particles sediment without agglomeration and that there are no significant changes in the particle size distribution.

Correlations between OD600 absorbances and the corresponding turbidity analyses at 25 ° and 90 ° indicate that OD600 is suitable for internal quality control regarding the intensity and batch consistency of turbidity.

The evaluation of the analysis results for the non-volatile beer compounds brought further insights. Increasing the pellet quantity correlated with a linear increase in the IBU and humulinones. There is also an increase in alpha acids and polyphenols, which show a trend for linear correlation. Iso-alpha acids decrease by up to 24 % at a dosage rate of 1500 g/hl. The timing of the dry-hop application has no significant impact on IBUs, alpha acids and humulinones. The influence on the iso-alpha acids is lower for hop applications in late stages of fermentation and maturation.

Increasing hop dosages lead to an increase in alcohol content and a decrease in real extract but do not show linear correlations. Dry-hopping in active fermentation results in higher alcohol and lower real extract. Cooling down to 5 °C precipitates yeast, inhibiting further metabolisation.

Every dry-hopping addition increases the pH, correlating with the quantity of hops added. The impact on the pH is greater with hop additions later in the process, which indicates less pH influence during active fermentation.

The investigations of this study proved that dry-hopping influences almost all aspects of the beer matrix. In particular, the influence on the turbidity and haze formation were closely examined and visualised to further understand the behaviour of dry-hopped beer.

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Appendix

Tables 3 and 4 show summaries of all results obtained from the analyses of beers produced for trial quantity (TQ) and trial timing (TT) at the Simon H. Steiner, Hopfen, research brewery. The reported values are means ±standard deviation of triplicate trials.

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