Comparison of Hazes in Freshly Bottled and Aged Beers by Multiple Angle Turbidimetry

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Abstract

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The hazes of freshly bottled and aged pale lager beers determined with the 12° and 90° dual angle laboratory hazemeters and 10° to 90° range photogoniometer were compared and evaluated. The instruments were standardised in EBC formazin units. In freshly bottled beer, the forward (12°–25°) haze values were smaller approximately by a factor of three than the nephelometric (90°) values which yielded 0.33 EBC units. In aged beer, the forward haze was greater than the nephelometric one. Whereas the aged beer showed the greatest and the fresh beer the lowest intensity of scattered light, the formazin suspension intensity was in between. Due to the standardisation of the beer scattered intensities by relation to that of formazin, the standardised nephelometric haze in non-aged beer was greater than the forward haze, and vice versa in aged beer. The greater forward than the nephelometric haze in aged beer was caused by the growth of haze particles above the mean size of formazin particles which was larger than 2 μm as confirmed by the particle size distribution measurement.

Keywords: beer; ageing; haze; multi-angle turbidimetry

It is well known that freshly filtered and packed beers are initially bright, but with time they become hazy due to the light scattering effects of chill and permanent haze particles which develop during the storage (Smythe *et al*. 2002; Sladký *et al*. 2004; Sladký 2005). The statement holds particularly for pale lager beer types packed in transparent bottles in which the haze or clarity of beer can be directly inspected and evaluated visually by the customer or consumer (Smythe *et al*. 2002; Clark & Bamforth 2007). Thus, the clarity or haze is a very important analytical parameter as well as a beer quality attribute (Mundy & Boley 1999; Smythe *et al*. 2002). The determination of haze in beer is particularly used for the control of the filtration and stabilisation technology and the determination and prediction of the shelf life of the final product (Klimowitz & Byrnes 1994; Clark & Bamforth 2007).

For the evaluation of the beer physical stability and prediction of shelf life, the brewing reference analytical methods (Analytica-EBC 1997) use almost universally the haze determination by light scattering at the angle of 90° relative to formazin suspension standard (Analytica-EBC 1997; Ebie *et al*. 2006). Besides the hazemeters or turbidimeters which determine the haze at scattered light angle either 90° (Buckee *et al*. 1986; Sladký *et al*. 2004) or 13° (Mundy & Boley 1999), there are at present in the current use the so called dual (scattering) angle hazemeters which enable the determination of haze in beer not only at the scattering angle of

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90° but also in the forward direction of scattered light, usually in the 11° to 25° range (Klimowitz & Byrnes 1994; Sladký 2005). The dual angle hazemeters have been proved to be very useful for the control and evaluation of filtration technology efficiency (Klimowitz & Byrnes 1994) because, when standardised by formazin suspension (Webster 1983; Morris 1987), they can detect more effectively the presence of coarse haze particles larger than several micrometers (e.g. yeast, filtration aids fragments etc.) in filtred beer (Morris 1984; Buckee *et al*. 1986).

However, except a few mentions (Klimowitz & Byrnes 1994; Sladký 2005), the use of dual angle turbidimetry for the analysis and evaluation of the beer physical or colloidal stability is hardly dealt with in the brewing literature, although it is well known that during the ageing process, the initially macromolecular and submicron beer haze particles can grow and form coarse flocks of super micrometer size (Morris 1984; Clark & Bamforth 2007). Experimental items of information would be useful for the determination and evaluation of the beer physical stability and ageing process generally by "non-destructive" optical techniques, i.e. by the optical measurements directly on beer samples in unopened commercial bottles, without the necessity of opening the bottles and pouring the foaming sample into the measuring cuvette. Such "nondestructive" types of optical techniques are particularly effective in the studies of the chill haze formation during the beer ageing process for an improved determination of both the beer and the flavour shelf life. This is because the chill haze particles could be, in undefined manner, irreversibly reformed or even destroyed by mixing and pouring during destructive measurements (Sladký 2005).

It was therefore interesting to determine the hazes in freshly bottled and aged beers with the 90°/12° dual angle laboratory hazemeters and, for the evaluation of results, to compare them with those determined by means of the innovated photogoniometer (a scanning hazemeter (Kratochvil 1987; Wyatt 1993; Jones 1999) in the non-standardised as well as formazin standardised modes and, as to the size, with the ("less fragile") permanent haze particles, determined in well aged beer samples by means of a laser particle counter. Because of consumers preference and for other practical reasons, a low level chill-proofed, all malt pale lager of typical Czech Pilsner type (Čejka *et al*. 2004) was chosen for the present study.

MATERIAL AND METHODS

Beer samples. Single brand (10% OG) beer, modestly stabilised for 3 months shelf life as read from the label, packed in 0.5 l bottles, was used in all experiments. The beer was a pale lager of typical Czech Pilsner type and composition (Čejka *et al*. 2004). A box of 20 bottles of the beer samples was purchased in a supermarket 5 days from bottling dated on the label. The samples measured on the day of purchase were assigned as fresh or as 0 month aged beer (M0). The haze values of the same, but aged samples, measured on the second, third, and sixth month from the purchase day, were assigned as M2, M3 and M6. The samples were aged at laboratory temperatures of 25 °C. At the age of 2 months, the samples were subjected to chilling in laboratory thermostat for 24 h at 0°C. At the end of chilling, their 0°C haze or the so called total haze after chilling (designed as M2 0°C sample series haze in Table 2 of the Results and Discussion section) was measured. After the measurement, the bottles with beer were spontaneously warmed for further ageing at 25°C without any other chilling.

Haze determination. For the determination and evaluation of fresh and aged beers haze, the 90° and 12° dual angle hazemeter type DATTS 2000 (Sladký 2005), was used, developed at the Department of Chemical Physics and Optics (Charles University in Prague, Czech Republic). The operating wave length of the hazemeter was 650 nm. The instrument was standardised according to (Analytica-EBC 1997) with formazin suspension in EBC units (EBC u.). The angular distribution of scattered light from bottled beer samples was performed by the on-purpose build photogoniometer (PG), developed at the authors laboratory. The instrument was essentially of the construction of Webster´s scanning hazemeter (Webster 1983) modified by improved opto-electronics and opto-mechanics.

For the purpose of the present study, the instrument was operated in the 10°–90° angular range of directions of the received scattered light beam. The operating wave length of the photogoniometer laser was 650 nm. For the measurement of total haze after chilling, the bottled beer samples were incubated at 0°C for 24 h and during the measurement were kept approximately at the same temperature. The long term photometric stability of both the DATTS hazemeter and the

Table 1. Nomenclature and symbols of determined beer haze values alternatively used throughout this study

Determined haze at scatter angle	Permanent or total haze at 25°C	Total haze after chilling or haze at 0°C
Forward 12° scatter or 12° haze	12H25 or 12H	90H0
Nephelometric or 90° haze	90H25 or 90H	12H0
Photogoniometric or PG° haze	PGH25 or PGH	PGH0

photogoniometer was better than ± 1% as tested by opal glass light scattering standard. The error of formazin standard suspension haze determination using the DATTS laboratory hazemeter directly on crowned commercial bottles was less than ± 2.5% of the measured EBC u. value. The reproducibility of the photogoniometer detected scattering diagrams was better than ± 2.5% in the non-standardised mode and better than ± 5% in the formazin standardised mode. The nomenclature and symbols of the beer haze values determined are summarised in Table 1.

Assessment of haze particles size distribution (PSD) and scattering cross section (SCS). The size distribution of the beer coarse haze particles was assessed by means of a simple and cost effective optical particle counter (mod. PC 2400, Chemtrac Systems Inc, Norcross, USA). The instrument was operated in the laser light beam blockage mode at the wave length of 780 nm. It counted haze particles larger than 2 micrometers in 1 ml sampling volume and distributed their sizes into eight classes: 2–5, 5–10, 10–15, 15–25, 25–50, 50–80, 80–100, and larger than 100 μm. The squared value of the approximate average diameter of the particles in each size class was used for the calculation of the relative values of haze particles SCS. According to the theory of light scattering (Kratochvil 1987), the total SCS was calculated as the product of the number and the relative average SCS of particles in each size class (Table 5).

RESULTS AND DISCUSSION

Fresh and aged beer hazes determined by dual angle laboratory hazemeter standardised with EBC formazin suspension

The results of the determination of fresh and aged beer hazes by dual angle laboratory hazemeter are summarised in Table 2. The data are the means of the measured haze values of 10 bottled samples from each age series rounded to the second decimal place. As can be seen from Table 2, the initial permanent haze values of fresh beer (12H25 and 90H25, M0) increased only slightly with the time of ageing (12H25 and 90H25, M2, M3 samples). Also, the ratio 12H25/90H25 increased moderately during the ageing of the samples at laboratory temperatures. However, the ratio of 12H0/90H0 of the M2 samples, i.e. total haze after chilling of 2-month-old samples crucially increased above one. After dispelling chill haze particles by warming, the 12H25/90H25 haze ratio returned almost to its laboratory temperature value and increased only slightly during the further month of ageing at 25°C to the end of the declared shelf life of the samples studied (Table 2, samples M3). During further 3 months of ageing following the shelf life period, however, the 12H25/90H25 permanent haze ratio (sample series M6, Table 2) increased above one, i.e. the M6 forward scatter permanent haze value was greater than its neph-

Table 2. Mean values (10 bottles) of the hazes determined by laboratory hazemeter on fresh and 2, 3 and 6 months aged beer samples M0, M2, M3, M6 at 25°C and of the M2 at 0°C after one day chilling from 25°C

Beer samples (age)	Temperature of the samples (°C)	Laboratory hazemeter		
		12H (EBC u.)	90H (EBC u.)	12H/90H
M0 (fresh)	25	0.10	0.31	0.323
M2 (2 months)	25	0.19	0.55	0.345
M2 (2 months)	0	3.15	0.87	3.62
M3 (3months)	25	0.38	0.97	0.392
M6 (6 months)	25	1.95	1.28	1.52

elometric counter partner. Similar effects in the aged beers were observed already by (Buckee *et al*. 1986) when they compared the haze response of the single angle Monitek 13° instrument with that obtained with 90° hazemeter (Morris 1984). Using the comparison of the beer haze with the haze of polystyrene latex suspensions of defined particle size, Morris (1984) ascribed the effect to the presence of various coarse haze particles in beer. Even before, the effect of the coarse haze particles on the forward to nephelometric haze ratio was clearly experimentally demonstrated by Webster (1983) by the use of the ab definitio experimental method of the scattering photogoniometry (Wyatt 1993; Klimowitz & Byrnes 1994; Jones 1999).

Angular distribution of light scattered from fresh and aged beer samples and 1 EBC formazin suspension measured by photogoniometer in non-standardized mode

In the past, the effect of particle size on the values of beer haze determined in nephelometric and forward direction of scattered light was discussed on general theoretical basis (Kratochvil 1987; Wyatt 1993). To demonstrate the effect experimentally, an innovated light scattering photogoniometer was used.

Figure 1 shows the relative intensity signals detected by the non-standardised photo-goniometer vs. the angle of scattered light (a scattering diagram) from 1 EBC u. formazin suspension, a fresh bottled sample, and 2- and 3-month aged samples (M2 and M3, respectively). In Figure 2 are compared scattering diagrams of the fresh (M0) and 6-month aged beer samples (M6) and 1 EBC u. formazin suspension. It can be seen from Figure 1 that the intensity of light scattered by beer haze particles was lower than in the case of 1 EBC u. formazin and that it increased with the age of beer to the levels that would exceed the level of the light scattered by 1 EBC u. formazin within the whole range of scanned angles (Figure 2, samples M0, M6 and 1 EBC u. formazin).

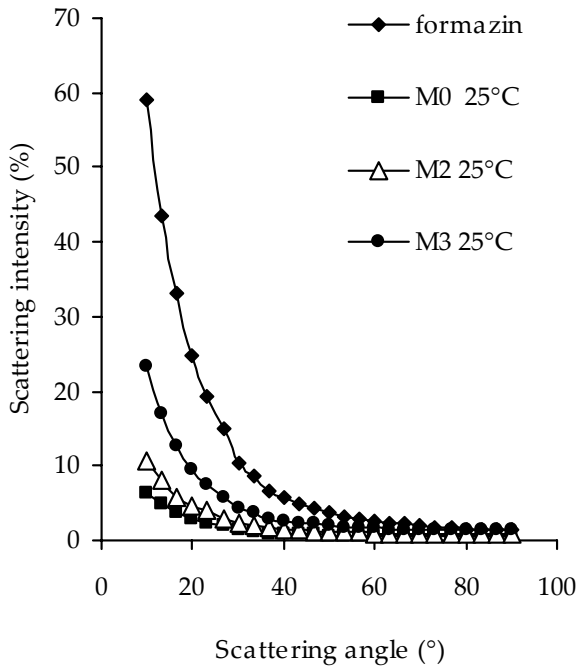

Figure 1. The relative scattering intensity vs. angle of scattered light (a scattering diagram) detected by the non-standardised photogoniometer on 1 EBC u. formazin suspension, fresh bottled samples (M0 25°C), and 2- and 3-month aged samples (M2 25°C and M3 25°C, respectively)

Comparison of fresh and aged beer hazes determined by EBC standardised photogoniometer and 12°/90° dual angle laboratory hazemeter

The scales of most hazemeters or turbidimeters are standardised, i.e. related to some light scattering standard suspension. A majority of brewing hazemeters are standardised or calibrated with formazin suspensions obtained by standard preparation procedures and of concentration units, in compliance with the standard reference methods of analyses (Analytica EBC 1997).

The standardisation of the light scattered signals from beer samples measured either at fixed angles, e.g. 12°and 90°, by a laboratory hazemeter or at variable angles by a photogoniometer is simply performed through the division by the signal from formazin suspension of the unit concentration, e.g. 1 EBC u. The results of the 1 EBC u. standardisation of the non-standardised scattering diagrams of the beer samples depicted in Figures 1 and 2 are shown in Figure 3 for comparison of standardised permanent (25°C) haze values vs. scattering angle of the M0, M3 and M6 samples, and in Figure 4 for the comparison of permanent (25°C) hazes vs. scattering angle of the M0 and M2 samples and the total haze values after chilling (0°C) vs. scattering angle. It is seen from Figure 3 that, whereas the permanent hazes 12H25 of the

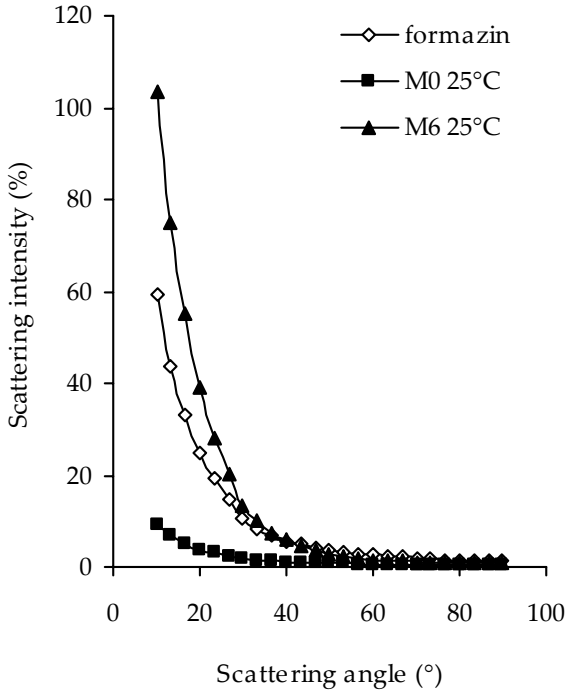

Figure 2. Scattering diagrams of 1 EBC u. formazin suspension, fresh bottled (M2 25°C), and 6 months aged samples (M6 25°C)

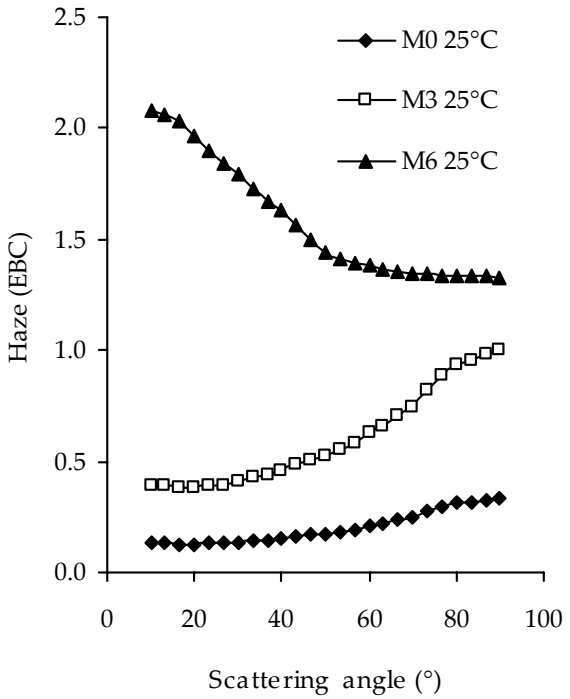

Figure 3. Standardised (1 EBC u. formazin) scattering diagrams of fresh bottled (M0 25°C), 3- and 6-month aged samples (M3 25°C and M6 25°C) permanent haze

samples M0 and M2 were still smaller than the 90H25, the corresponding permanent haze values of the samples M6, whose age exceeded twice the shelf life (3 months), showed the opposite, i.e. the forward scatter permanent haze of these samples was greater than the nephelometric one. Such a situation had already been observed with the formazin standardised values of total haze after chilling 2-month-old samples after one day of chilling at 0°C, whereas the permanent haze values of these samples were still the opposite, i.e. 12H25 was smaller than 90H25 as can be seen from the comparison of the standardised haze diagrams of the M2 25°C and M2 0°C samples. Essentially the same results were obtained with formazin calibrated laboratory hazemeter at 12° and 90° angles as shown for comparison in Table 3.

Coarse haze particles size distribution of examined beer samples

The growth of hazes determined with the 12°/90° hazemeter as well as photogonimeter on the bottled beer samples with increased age indicates, in accordance with the theory of light scattering (Kra-

Table 3. Comparison of 90° and 12° permanent and chilled hazes in fresh (M0) and 2 months (M2) to 6 months (M6) aged beer samples

Beer samples (age)	Temperature of the samples (°C)	Haze 12H (EBC u.)		Haze 90H (EBC u.)	
		laboratory hazemeter	photogoniometer	laboratory hazemeter	photogoniometer
M0 (fresh)	25	0.10	0.12	0.33	0.35
M2 (2 months)	25	0.19	0.21	0.55	0.60
M2 (2 months)	0	3.15	2.83	0.87	0.81
M3 (3 months)	25	0.38	0.41	0.97	1.03
M6 (6 months)	25	1.95	2.07	1.28	1.37

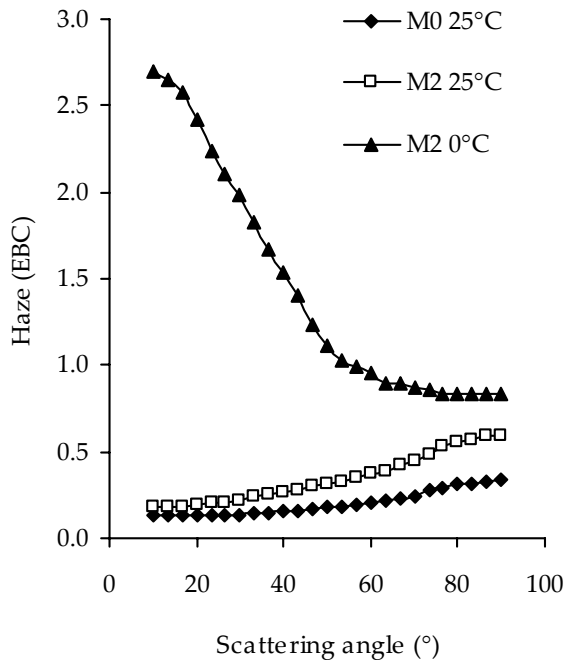

Figure 4. Standardised (1 EBC u. formazin) scattering diagrams of fresh bottled (M0) and 2-month aged (M2) beer permanent haze (M0 25°C and M2 25°C) and total haze after chilling 2-month aged samples (M2 0°C)

TOCHVIL 1987; JONES 1999), a consecutive growth of the mean size of haze particles during the beer ageing processes (MORRIS 1984; SLADKY 2005). On the basis of the beer haze determination by light scattering measurements, standardised by formazin suspension, it can be anticipated that the forward haze values greater than nephelometric ones are due to the presence of coarse haze particles larger than 2.5 μm, which is approximately the mean size of the formazin standard suspension particles as confirmed by recent size distribution measurements (EBIE *et al*. 2006). To evaluate these findings quantitatively on the aged beer samples examined, the size distribution of coarse permanent haze particles in aged beer samples larger than 2 μm was measured using a cost effective laser particle counter. The measured (counts per 1 ml) particle size distribution (PSD) data on beer, linearly normalised to the nephelometric haze level of 0.33 EBC units in freshly bottled beer samples, were compared in Table 4 with those of formazin suspension, and with the PSD of the residual coarse haze particles in clear water prepared by osmosis filtration. The highest amount of particles was found in the 2–5 μm class with all of the samples compared. This could be expected for formazin suspension with PSD mean in this interval. However, the 2–5 μm to 5–10 μm particle size class count ratio of formazin is much greater than those of the other samples (Table 4, last row) indicating that they contained relatively large amounts of coarse haze particles in the 5–10 μm size class. Whereas the contribution of these particles to the forward haze in filtred water and fresh beer is relatively small, it is quite great in aged beers. This is confirmed by the distributions of the particle SCS shown in Table 5, and particularly by their sums, which correlate with non-normalised levels of scattered light shown in Figure 1 and 2, in agreement with the theory of light scattering.

When the total SCS values are normalized to the forward 12H level 0.10 EBC units of fresh (bright)

Table 4. Comparison of distributions of haze particles greater than 2 μm in studied samples, normalised linearly to the 0.33 EBC u. nephelometric haze level of fresh beer

Particle size class (μm)	Clear water 12H = 90H = 0.01 EBC u.	Formazin 12H = 90H = 0.33 EBC u.	Fresh beer (M0) 12H = 0.10 EBC u. 90H = 0.33 EBC u.	Aged beer (26% M6) 12H = 0.50 EBC u. 90H = 0.33 EBC u.
2–5	33.1	1558	307	1721
5–10	5.7	45.7	57.5	1368
10 -15	3.3	13.4	14.8	125
15–25	1.6	11.9	6.3	64
25–50	0.25	1.95	2.5	27
50–75	0	0.17	0	13
75–100	0	0	0	3
> 100	0	0	0	0
Ratio of 2–5 to 5–10 class	5.7	34	5.3	1.26

Table 5. Comparison of the haze particles relative scattering cross sections and their sums for the nominally zero haze clear water and formazin suspension, fresh (i.e. bright) and aged (hazy) beer at the 0.33 EBC u. reference level of the nephelometric permanent haze

Particle size class (μm)	Relative scattering cross section (approx.)	Scattering cross section distributions of the sample particles			
		clear water 12H = 90H = 0.01 EBC u.	formazin 12H = 90H = 0.33 EBC u.	fresh beer M0 12H = 0.10 EBC u. 90H = 0.33 EBC u.	aged beer M6(26%) 12H = 0.50 EBC u. 90H = 0.33 EBC u.
2–5	2	132	6 232	1 228	6 884
5–10	4	114	914	1 150	13 180
10 -15	6	165	1 025	740	4 800
15–25	10	160	1 190	630	4 100
25–50	15	56	439	562.5	1 575
50–75	30	0	153	0	2 520
75–100	40	0	0	0	0
> 100	50	0	0	0	0
Sum	–	628	9 953	4 311	33 059
Approx. SCS (normalised to 12H = 0.1 EBC level)		188	9 953	1 293	9 918

beer, the forward hazes of the measured samples correlate naturally better with the forward haze values than with the nephelometric ones as can be seen in Table 6. For a better quantitative correlation between the size of the coarse haze particles and the values of the 12H and 90H hazes in fresh and aged beers, it would be necessary to determine more values over a wider range of levels taking into account e.g. multiple scattering effects at larger size and number of particles. However, the results obtained and the estimations performed above indicate, although very approximately, that when the number or total SCS of coarse haze particles greater than 2 μm exceeds, at the same haze level that of formazin, then the forward 12H haze exceeds the nephelometric 90H one in the measured sample. If one supposes that the light scattering properties of the chill haze particles are approximatively the same as those of permanent haze particles, then the conclusion similar to that above can be applied for the population of the beer chill haze particles, which was qualitatively confirmed by "destructive" electrical counting techniques (Morris 1984). These findings are very important for the estimates of some average particle size of chill haze in bottled beer samples without their excessive thermal and/or mechanical destruction due to opening the bottle for the preparation of samples for measurement.

CONCLUSIONS

The methods of haze determination by means of 12° and 90° dual angle turbidimetry, 10° to 90° photogoniometry, and laser photometric particle size discrimination and counting have been used to compare and evaluate the effect of the particle

Table 6. Correlation of the SCS normalized to the level of the fresh beer forward scatter haze 12H = 0.10 EBC u. i.e. the total SCS values (except that of formazin) divided by the factor 0.33

Total scattering cross section (relative units) with EBC u. haze	Clear water	Fresh beer	Formazin standard	Aged beer (26%)	Correlation
Scattering cross section (approx.)	188	1293	9953	9918	1
Measured 12H (EBC u.)	0.01	0.1	1	0.50	0.89
Measured 90H (EBC u.)	0.01	0.33	1	0.33	0.71

size upon the haze values measured in fresh and aged beers. When the haze measuring instruments were standardised in EBC formazin units, then in filtred and freshly bottled beer the forward (12°) haze values were lower approximately by a factor three than the nephelometric (90°) values which yielded 0.33 EBC u. In aged beer, the forward chill and later on the permanent haze, too, was greater than the nephelometric one. The observed effects were elucidated by comparison of the intensity of scattered light from formazin and beer samples of progressive age. Whereas the aged beer showed the highest and the fresh beer the lowest intensity of scattered light, the formazin suspension intensity was in between. Due to the standardisation of the beer scattered intensities by relation to those of formazin, the standardised nephelometric haze in non-aged beer was greater than the forward haze and vice versa in aged beer. The forward haze greater than the nephelometric one in aged beer was caused by the growth of haze particles over the mean size of formazin particles which was larger than 2 micrometers as confirmed by laser particle counting and size distribution measurement.

The results obtained can be particularly used for an improved and experimentally better controlled ("nondestructive") evaluation of beer ageing kinetics directly in unopened commercial bottles without physical (mechanical) as well as chemical (oxidative) perturbation of the beer haze active particle system by excessive sampling procedures.

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