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Rapid method to assess the colloidal haze stability of beer

Due to a rising globalization in food and beverage industry, consumers expect a long chemical-physical beer stability without an appearance of haze. The analysis of the chemical-physical stability, however, often represents a long process when the classic forced aging test is applied. In comparison, alcohol-chill or formaldehyde test can be performed in a shorter analysis time, but barely gives information on the real beer stability. For this reason, the aim of this work was to develop a rapid measurement method determining colloidal stability. To accelerate the haze formation, an oxidizer was added into beer and 3 temperature changes of 0 and 60 °C were carried out. An optimal amount of 1 mL hydrogen peroxide per 500 mL beer (0.06 %) was determined. After 26 h, the haze increase could be examined using a scattering light angle measuring device. The comparison of this haze increase and the warm days of the classic 0/60 °C forced aging test resulted in an exponential function with $R^2 = 0.87$ ($n = 38$, samples of 10 breweries), whereas 3 non-stabilized beer samples had significantly higher haze increases. Furthermore, stability could be classified comparing warm days and haze increase in low stability (0–4 warm days): > 1 EBC haze increase, medium stability (5–8 warm days): 0.1–1 EBC haze increase and high stability (above 8 warm days): < 0.1 EBC haze increase. Due to the good comparability, the new method gives fast and practical information on the haze stability of beer within two working days.

Descriptors: colloidal stability, beer stabilization, forcing test

1 Introduction

Beyond the best before date, the consumer expects a clear product without flakes or haze, not only in lager beer [20]. This feature is of particular interest to export beers, as the consumer usually does not differentiate between various types of haze but associates it with microbiological spoilage. For this reason, preventing haze and the associated prediction of this feature is a key issue in the brewing industry.

Due to the complex composition and annual variations in raw materials, beer haze is still an unsolved problem in breweries. Although there are now ways to remove haze inducing substances from the beer through stabilization, storage and filtration selectively, nevertheless, despite a good brewing practice, haze can occur anyway [2]. This was also evident in the end of 2018. Reasons for these haze formations can certainly found in the change of the 2017 barley harvest to the 2018 with the special processing criteria like higher gelatinization temperatures and nitrogen contents due to the long hot summer in central Europe, which in many breweries led to increases in volume and sales but shortened the storage times additionally.

To date, no general differences in the composition of these haze types have been found [21, 22]. Beer haze is composed of a mixture of complex molecules with a large share of haze sensitive proteins or polypeptides (rich in proline) and polyphenols of different polymerization, which are subjected to a natural movement (Brownian molecular motion) [17, 21]. In addition to proteins and polyphenols, oligosaccharides, high molecular weight polysaccharides or associations of polypeptides and minerals may be involved in the haze formation [2, 17]. Delvaux et al. [8] identified the complex haze in Belgian white beer as proteins (7–74 %), polyphenols (1.1–7.4 %), degraded starch (9–65 %) and β-glucans (0–0.5 %). Minor components were arabinoxylans, metal ions and yeast cell wall mannose residues. In general two haze types can be distinguished: the permanent haze and the reversible chill haze that occurs when the beer is cooled and disappears when heated.

Depending on the ingredients different haze structures or forms can be visible. Typical haze structures range from dispersed haze and precipitates to flakes or “bits” [2]. Visible haze arise due to a collision of the particles that leads to a gradual coarsening of the degree of dispersity. This formation of visible haze particles is improved by temperature changes, shear forces (Brownian molecular force) or movement, oxidation, the presence of catalysts like heavy metal ions, light and oxygen [1, 4, 10, 18, 22]. In order to investigate the haze stability of the beer, these factors can be used to accelerate haze formation.

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To measure haze stability of beers, several tests are available in practice. The best known and most used test in brewing industry is the forced aging test with different modifications [3, 9, 11, 19]. In general this accelerated shelf life tests simulate temperature changes between 0 and 40 or 60 °C, resulting in a haze formation over time [11, 19]. A major disadvantage of this method, despite the good comparability to long-term stability, are the long analysis times of several days or even weeks. Another direct measurement method is the alcohol-chill test according to *Chapon* [5, 6, 15] that is based on the prevention of solubility of the chill haze inducing substances by the addition of alcohol. Indirect measurement methods including Esbach reaction test, formaldehyde test and ammonium sulphate precipitation limit predict only the impact of one haze inducing substance. Table 1 provides an overview of the most important prediction methods for the colloidal haze stability as well as the measurement principles and the respective advantages and disadvantages.

Table 1 **Tests for the prediction of haze stability in beer according to MEBAK Wort, Beer and Beer-based beverages [7, 11]**

	Name and method number	Principle	Advantage	Disadvantage
Direct measurement method	Forced aging (2.14.2.1)	forced aging of the samples in a water bath at 0/40 °C or 60 °C for 24 h	realistic result including all turbidity factors	long analysis time
	Alcohol-chill haze (ACT) according to Chapon (2.14.2.2)	solubility of the haze inducing substances of chill haze is prevented by the addition of alcohol	rapid test; allows statement about the expected permanent haze (indirect)	haze measurement refers only to the reversible haze
Indirect measurement method	Formaldehyde test (2.14.2.3)	forced aging is accelerated to 24 h by addition of formaldehyde solution	provides fast information about expected haze stability	low information for stabilized beers (especially when using PVPP)
	Precipitation with ammonium sulphate (2.14.2.4)	by adding ammonium sulphate, protein is precipitated	fast clue about expected beer stability	only information about the influence of protein fractions
	Esbach reaction test (2.14.2.5)	by adding Esbach reagent, high molecular weight protein is precipitated	indication of chemical-physical stability	only information about the influence of high-molecular protein fractions

Since early knowledge of colloidal stability is important for breweries and existing methods do not provide reliable information or have very long analysis times, the aim of this paper was the development of a simple, rapid method with no need in special laboratory instrumentation that is comparable to the classic forced aging test with temperature cycles (0/60 °C). The test should provide practical information without detailed investigation of the beer composition. This would not only save costs due to faulty productions with low haze stability or low foam stability and fullness due to over-stabilization, but in additional cost savings for stabilizers, cleaning supplies and water consumption.

Table 2 **Examined beer samples with starting haze in 90°- and 25°-scattering angle**

Beer style	Sample number	Average starting haze at 90°-scattering angle	Average starting haze at 25°-scattering angle
Pale lager	11	0.26 ± 0.17	0.08 ± 0.04
Pilsener	17	0.34 ± 0.21	0.11 ± 0.06
Pale export	5	0.47 ± 0.26	0.13 ± 0.05
Alcohol-free pale lager	3	0.15 ± 0.01	0.10 ± 0.03
Crystal wheat beer	2	0.86 ± 0.06	0.43 ± 0.26
Octoberfest beer	2	0.74 ± 0.22	0.11 ± 0.03
Beer mix with lemonade	1	0.34	0.13

2 Material and methods

2.1 Beer samples

41 different beer samples from 10 breweries (see Table 2) were used to develop the new rapid method for the elaboration of the new haze test and comparison with existing methods. All beer samples had a starting haze smaller 1 EBC at 90° and 25°-scattering angle. The beer haze was measured with the LabScat 2 (Sigrist, Ennetbürgen, Swiss).

2.2 Beer composition

Total polyphenols (Analytica-EBC 9.11), anthocyanogens (MEBAK Wort, Beer and Beer-based beverages 2.16.2) and total nitrogen content (Analytica-EBC 9.9.2) were determined in all beer samples in triplicate [12].

2.3 Determination of colloidal haze stability

2.3.1 Forced aging test according to MEBAK

A classic forced aging test according to MEBAK Wort, Beer and Beer-based beverages 2.14.2.1 was performed [11]. The starting haze of the sample was measured, followed by an incubated for 24 h at 60 °C and a subsequent incubation at 0 °C for 24 h. A water bath of the company Julabo (Julabo GmbH, Seelbach, Germany) was used for the incubation of the samples. Haze increase of the sample was subsequently measured at the end of the 0 °C incubation using Sigrist LabScat 2 (Sigrist-Photometer Gmbh, Unterpleichfeld, Germany). Aging was stopped after a haze

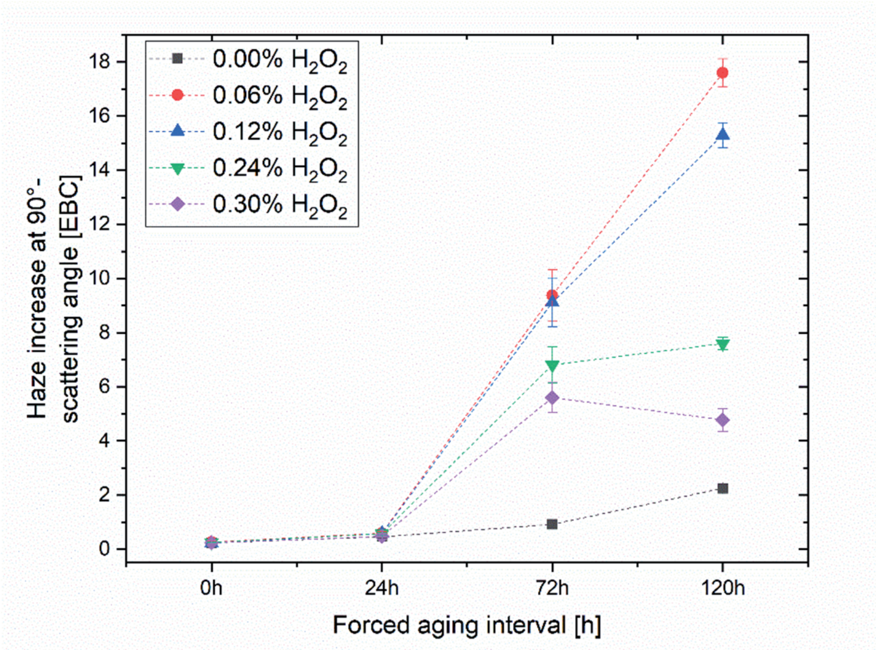

Fig. 1 Investigation of the optimum amount of the oxidizer hydrogen peroxide with an aging interval of 12 h at 60 respectively 0 °C to determine the largest haze increase

increase of 2 EBC at 90°-scattering angle and the number of warm days were determined.

2.3.2 New rapid forced aging test

The main aim of the new forced aging test was a reduction of analysis time taking into account as many influencing factors on haze formation as possible. Basis of the method was a forced aging test with temperature cycles, which should be modified to accelerate the haze formation and minimize the reaction time. To optimize the reaction conditions aging temperature, length of incubation and impact of oxygen (oxidation) should be tested [1, 4, 18, 22]. Higher temperatures were tested by *Rice* et al. [18] (70 °C as used in wine) and *Schild* et al. [19] (80 °C), however no haze acceleration could be determined. On the contrary, the repeatability of the experiments decreased sharply [19]. Another method was developed by Harp, whereas this test uses an incubation at 37 °C for 4 weeks with a subsequent cooling to 0 °C for 8 h [3]. In the EBC method from 1963 this time was shortened to 8 days due to an increase in temperature to 60 °C and a subsequent cooling to 0 °C for 24 h. In addition different cycling methods at 37/ 40/ 60 °C and 0 °C for 24 h each can be found in literature [13]. Since a fast haze formation was the aim of the new method, a temperature cycle of 0/ 60 °C was chosen. The aging of the samples was carried out in the water bath Pro RP 3035 (Lauda Dr. R. Wobser Gmbh & Co. KG, Germany). In order to further accelerate haze formation, a defined oxidation of the samples should be tested. For this reason, hydrogen peroxide (30 %, Sigma-Aldrich, Germany) was added into the beer. The investigation of the optimal amount of hydrogen peroxide was performed in a 12 h temperature cycle between 0 and 60 °C. 1 to 5 mL hydrogen peroxide per 500 mL beer (0.06 – 0.30 %) were added into a sample and haze formation was examined after 24, 72 and 120 h (see Fig. 1).

The blank sample had almost no increase in haze over the investigation period. This sample was exactly treated like the other samples with an addition of hydrogen peroxide (e.g. bottle opened, decanted and filled in the test bottles). In comparison, the addition of 1 ml of hydrogen peroxide (0.06 %) resulted in a haze increase of 8 EBC after 72 h. The addition of higher concentrations does not accelerate, but reduce the haze formation. For this reason, a concentration of 0.06 % or 1 mL of 30 % hydrogen peroxide per 500 mL was used for the new methodology. It should be noted that hydrogen peroxide has a limited shelf life and therefore the concentration should be checked regularly.

Besides the temperature and the addition of an oxidizer, the time and length of cycle of the forced aging should be optimized. Thus, 5, 7 and 9 temperature changes with 1, 2 or 3 h were tested (see Fig. 2). It was found that a longer temperature impact resulted in an increase in haze. A high number of temperature changes with a shorter time had a lower impact on haze formation. In order to obtain rapid information on the haze stability of the sample, 5 temperature changes with a 3-hour exposure time and a total analysis time of 26 h were selected for the further experiments.

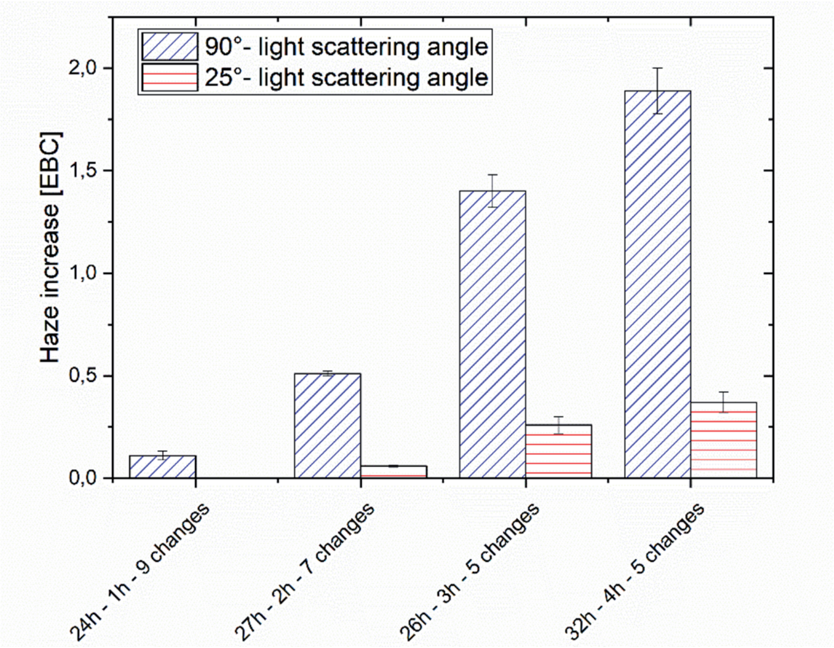

Fig. 2 Investigation of temperature interval and number of temperature changes of the new forced aging test at dosage of 0.06 % hydrogen peroxide as oxidizing agent determined as mean haze increase (n = 3) at a scattering angle of 90° and 25°

In addition, the cooling performance of different bottle sizes (200 and 500 mL) from 60 to 0 °C of the samples in the water bath was investigated in order to ensure the longest possible temperature exposure. In comparison to the water bath, the 200 mL bottle had a delay in cooling of 10 min, whereas the 500 mL bottle had about 60 min (data not shown). While the 500 mL bottle, which corresponded to the size of most of the original sampled bottles, deviates on average 8.3 °C from the current temperature of the water bath, the difference between water bath and 200 mL bottle was only 3.7 °C on average. To accelerate haze formation, 200 mL white glass U-lock bottles were used. In order to minimize the uncontrolled oxygen uptake during transfer, the samples were cooled to 0 °C and decanted slowly and foam-free. All experiments were carried out in triplicate to determine the repeatability of the new test.

The modifications described above result in the following method instruction for the new forced aging test: 0.4 mL of hydrogen peroxide were added into 200 mL sample. Haze of the sample was measured before and after addition of hydrogen peroxide. Incubation was started at 60 °C for 3 h with 5 temperature changes between 60 and 0 °C. After 26 h reaction time, haze of the sample was measured using Sigrist LabScat 2 and increase in comparison to starting haze was calculated.

2.4 Statistics

The statistical analysis was carried out with OriginPro 2018b (OriginLab Corporation, Northhampton, USA).

3 Results and discussion

To examine the comparability of the two colloidal stability prediction methods, 41 beers from 10 different breweries were used in order to have a wide range in haze stability and different applied stabilization methods. These samples were analysed on protein, polyphenol and anthocyanogen content. The total soluble nitrogen content had an average of 815.7 mg/L (n = 41) with a range between 563 – 1164 mg/L. Polyphenols ranged between 59 – 277 mg/L (average: 155.8 mg/L, n = 41) and anthocyanogens between 12 – 129 mg/L (average: 50.1 mg/L, n = 41). Anthocyanogen and polyphenol content correlated significantly (r = 0.732 P < 0.001).

In addition to the chemical composition, classic forced aging test was performed. Starting haze values of the beer samples can be found in table 2. After forced aging, the samples had an average haze increase of 2.67 EBC at 90°-scattering angle. Maximum haze increase was found in a pilsner beer with 5.79 EBC after one temperature change (one warm day). The average analysis time was determined with 13.6 days, which resulted in an average of 6.8 warm days (range: 1 – 22 warm days, n = 41). The measured warm days correlated significantly to nitrogen content (r = –0.520, P < 0.001), polyphenol content (r = –0.759, P < 0.001) and anthocyanogens (r = –0.604, P < 0.001).

Compared to the classic forced aging test, the new experimental set-up requires only 26 h to obtain practical information on haze stability. Hydrogen peroxide was added to all 41 samples and stability was determined after 5 temperature changes between 60 and 0 °C. The haze increase ranged between 0 and 9.0 EBC with an average value of 1.56 EBC. A good repeatability of the haze increase could be achieved with an average standard deviation of 0.09 EBC (range: 0.007 – 0.369 EBC) and a confidence interval of 0.028 %. Between the haze increase of the new test and the warm days of the classic forced aging test, a significant correlation r = –0.643 (P<0.001) could be determined. In addition, the haze increases of both tests at the end of aging correlated with r = 0.770, P < 0.001.With regard to the chemical composition of the beers, a significant correlation between haze increase of the new method and the total polyphenol content (r = 0.720, P < 0.001) could be achieved.

The tested beer samples were stabilized on both polyphenol (PVPP) or protein (silica gel) side as well as combinations. In addition, there were beers from 2 breweries that were not stabilized as a reference. These three samples had very high haze increases (>4.0 EBC 90°-scattering angle) in the classic forced aging test with two respectively three warm days. Furthermore, the protein contents were above 1000 mg/L and the polyphenol contents above 200 mg/L. Since the experimental conditions of the new test were very extreme, these non-stabilized samples had a haze increase between 5.3 – 9.0 EBC at 90° scattering angle, but two or three warm days in classic forced aging test. This shows that the new test gives no useful information on the haze stability of non-stabilized beer. Due to this high variation, these three values were excluded from further consideration. Figure 3

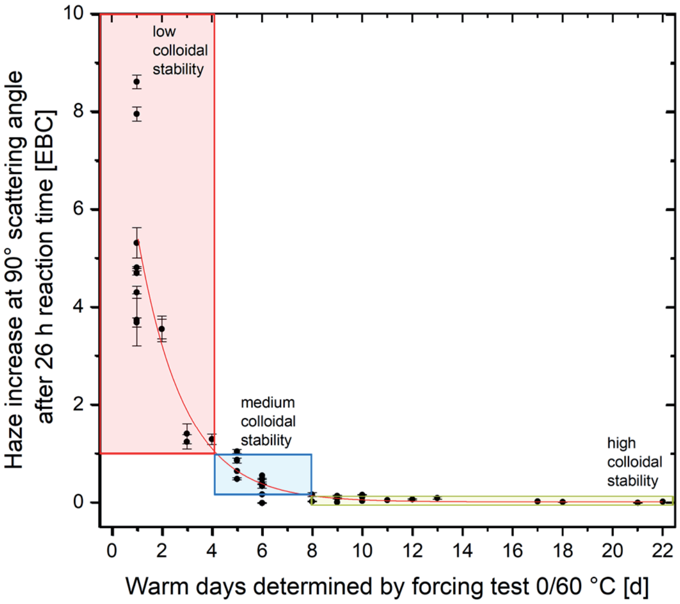

Fig. 3 **Comparison between the mean value of warm days of the forced aging test 0/60 °C (n = 3) and the mean haze increase (n = 3) of the new method of 38 beer samples as well as classification of the haze stability**

Table 3 **Assessment of colloidal stability with associated turbidity increase of the new method and comparison to the warm days of the classical forced aging test**

Colloidal stability and local distribution of beer	Haze stability in warm days classic forced aging test	Haze increase of new stability test, 90°-scattering angle [EBC]
Low – regional distribution	0–4	>1
Medium – nationwide distribution	4–8	0.1–1
High – export beer	>8	<0.1

shows the comparison of the mean haze increase (n = 3) of the 38 beer samples in comparison to their mean warm days (n = 3) in the classic forced aging test.

The data comparison resulted in an exponential equation ($y = 9.26 \cdot \exp(-x/1.85) + 0.012$) with $R^2 = 0.87$. Based on literature data [14, 16] an assessment of colloidal haze stability according to the warm days of classic forced aging test can be applied and compared to the haze increase of the new test (see Fig. 3 and Table 3). Table 3 shows the classification of the measurement results to assess the colloidal haze stability of beer samples.

According to the recommended local beer distribution, haze stability could be divided into three ranges: a low haze stability with 0–4 warm days in classic forced aging test, a medium haze stability with 4–8 warm days and a high haze stability above 8 warm days [14, 16]. Comparing these ranges to the measured data, a haze increase of more than 1 EBC for a low haze stability could be observed. Medium haze stability range had an increase between 0.1–1 EBC and high haze stability range an increase below 0.1 EBC. Only two of the tested samples (5.3 %) were small outliers from this stability assessment. An Anova (Tukey test) with repeated measurements was performed with the raw data of haze increases observing significant differences between these three areas. Thus, the haze stability can be assessed quickly (26 h) and easily by means of the new method.

Since the aim of the new haze stability method was to speed up haze formation to accelerate the assessment of haze stability, several steps in the forced aging test methodology had to be changed. The dosage of hydrogen peroxide allows rapid haze formation. According to *Uchida* and *Ono* [23, 24], oxygen radicals are formed during the aging of beer, which deteriorate the colloidal stability. To what extent this natural aging process can be replaced by the addition of hydrogen peroxide is questionable. Nevertheless, the aging and haze formation could be significantly accelerated. In addition, experiments must be performed to check for a possible comparability with natural haze formation. Non-stabilized beers, however, had very large deviations from the determined warm days and thus cannot be characterized by the method. This may occur due to the very high polyphenol concentration in these samples and their associated polymerization due to the impact of oxygen. In addition, it was noticeable that in these samples the haze in 25° scattering angle after reaction with hydrogen peroxide was significantly higher (between 1.7 and 3.6 EBC – otherwise never greater than 1.5 EBC). This suggests that the extreme reaction conditions of the new test build up very large particles in non-stabilized samples, which are not detectable with the 90° haze measurement. For this reason, a settlement of the two haze angles should be considered in order to be able to characterize non-stabilized beers.

Compared to the classic forced aging test, a time saving of a maximum of 41 days was achieved. Even for samples with only one warm day haze stability, the analysis time was halved. Another problem of the classic forced aging test is the termination of the measurement above a haze of 2 EBC. Even in our experiments, the beer samples often had a much higher haze than 2 EBC, which suggests on a lower stability as determined. These measurement inaccuracy could result in small deviations from the new method.

The adjustment of the forced aging intervals resulted in a further acceleration of haze formation. Even if the extension of the interval to 4 h would have resulted in a further acceleration of haze formation, an interval of 3 h allowed an analysis time of 26 h and thus a result on the next working day. In addition, the decrease of the sample volume had a positive impact on the analysis time. Although if a sample transfer into the 200 mL bottles may result in another oxygen uptake. However, it turned out that this error is negligible due to the extreme experimental conditions in terms of oxygen. The classification into 3 stability ranges allows a quick and useful information on the beer haze stability. According to Schild et al. [19] a stability of 6 warm days in classic 0/60 °C forced aging test is sufficient for a beer stability of 12 months. In comparison Meier [14] stated that one warm day at 0/60 °C corresponds to approx. 30–60 days in reality. This conversion heavily depends on the beer type and composition as well as the applied stabilization. With an average of 45 days, 8 warm days would be necessary for a stability of one year. In the new stability test, this corresponds to no increase in haze (< 0.1 EBC). Thus, a high stability of the samples can be assumed, if no haze increase is measurable after 26 h.

4 Conclusion

The new test is a rapid and easy way to predict the haze stability of beer. A good comparability to the classic forced aging test (0/60 °C) with a significant reduction in analysis time was found. It could be shown that the results are independent of the brewery or beer type. Thus, the test can be used by all breweries regardless of the type of stabilization or filtration. Only non-stabilized beer samples did not produce useful results. The changeover to smaller sample volumes allows both the examination of filled containers or bottles and samples from the bright beer tank, so that the haze stability can be determined before bottling. This would permit breweries to identify particularly stable batches for export. Furthermore, the methodology can be easily adapted to other temperatures. The new test requires no excessive time, labour, material equipment or instrumentation.

Although current methods such as the classic forced aging test provide good knowledge on haze stability, they require too much analysis time to identify deviant samples. In comparison, indirect methods with a shorter analysis time only consider one component involved in the haze formation and thus do not give an overall picture on the haze potential. In order to identify batches with a high stability or to optimally adjust the stabilization and avoid over-stabilization, the new rapid test can be easily applied. Furthermore, the method can be used to control the operation and function of stabilizers or to compare different stabilizing agents.

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