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ORIGINAL PAPER

Effects on beer colloidal stability of full-scale brewing with adjuncts, enzymes, and finings

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Abstract

This study investigated the effects on beer colloidal stability of full-scale brewing with adjuncts, enzymes, and finings. Industrial lager beers were produced solely from barley malt or from barley malt with adjuncts (corn grist and starch syrup or unmalted barley). Various stabilization aids were also used (silica gel, PVPP, proline-specific endoprotease, carrageenan). Predictive shelf-life tests were conducted. We analyzed the content of compounds (proteins and polyphenols) generally related to beer colloidal stability. The results show that the haze-forming potential of the beer during storage can be evaluated based on the coagulable nitrogen content (high molecular weight proteins), rather than the total nitrogen content and polyphenol content. A very strong and statistically significant negative correlation was observed between the concentration of coagulable nitrogen and beer colloidal stability. When brewing was conducted with 49% barley raw material and exogenous proteases, especially proline-specific endoprotease, the coagulable nitrogen content fell and beer colloidal stability improved. The use of corn grist and starch syrup as up to 40% of the total grist resulted in a 30% longer physical shelf life compared to the all-malt beer.

Keywords Beer · Colloidal stability · Adjuncts · Enzymes · Shelf life · Haze

Introduction

Beer is traditionally produced using only four ingredients: water, barley malt, yeast, and hops. Nowadays, barley malt is often partially replaced with liquid and/or solid adjuncts, such as unmalted barley, corn, or starch syrups. Adjuncts provide a cheaper source of extract, and may also contribute to improve beer colloidal stability [1, 2]. The addition of exogenous enzymes solutions is also commonly practiced in brewing, particularly when the use of adjuncts causes enzyme deficiency due to the lack of germination during malting [3, 4]. Exogenous proteases and β-glucanases are the most commonly used commercial brewing enzymes for brewing with unmalted raw materials. Exogenous proteases provide more free amino nitrogen for yeast nutrition. β-glucanases are used to reduce wort viscosity (improving lautering and wort filtration). The use of industrial proteolytic enzymes such as prolyl endopeptidase from *Aspergillus niger* has been associated with less susceptibility to turbidity [5].

The addition of stabilization aids such as silica gel or polyvinylopolypyrrolidine (PVPP) during beer filtration, or of carrageenan at the stage of wort boiling, has been reported to bring positive effects on beer colloidal stability and unfiltered beer clarity [5–9]. The mechanism of action by silica gel is based on the adsorption of proteins. The mechanism of action by PVPP is based on the adsorption of polyphenols. Thus, the use of silica and PVPP facilitates the removal of many haze-active compounds during beer filtration. The use of carrageenan supports flocculation and subsequent elimination of coagulable nitrogen (high molecular weight proteins) from the wort. It is estimated that without these adjuncts the colloidal stability of beer made using only traditional feedstocks cannot be maintained for more than three months of storage [10].

Haze formation can be caused by several classes of beer constituents originating from malt, adjuncts, or

Contribution for the Special Issue: Thechemistry behind malt and beer production – from raw material toproduct quality.

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hops. These include primarily proteins/polypeptides, polyphenols, and glucans [11]. Proline-rich proteins of the prolamin fraction (hordeins) ranging in size from 15 to 30 kDa have been identified as haze-active proteins that are particularly involved in protein–polyphenol interaction, resulting in the formation of a colloidal precipitate [5, 12, 13]. It can be inferred that the risk of haze formation in beer may be reduced by the selection of malt/adjuncts with a lower hordein/total nitrogen content or by the use of a proline-specific protease during mashing or fermentation. On the other hand, high molecular weight β-glucans found in the cell walls of barley grains have been reported to impede wort filtration, which may ultimately cause non-biological haze formation during storage [14]. Low β-glucan levels, such as are generally obtained when brewing all-malt beers, are thought to promote longer shelf life. The addition of unmalted barley may lead to haze problems, as a consequence of cell walls not being initially digested during malting. If there is no or inadequate enzyme treatment during the mashing process, beer haze may furthermore develop from non-hydrolyzed residual starch or excess coagulable nitrogen (high molecular weight proteins).

The visual quality of a beverage is of great importance to consumers. Any visible haze in light lager-style beers (turbidity level of more than 2 EBC (European Brewery Convention) units) [15] is often identified with microbial contamination [16]. Therefore brewers are required to produce consistent quality beers, exhibiting no physical changes across the whole shelf life. Beer, however, is an inherently unstable fermented beverage [17]. Its physicochemical properties such as clarity and its overall quality gradually deteriorate during storage, especially under poor storage conditions such as elevated temperature, movement, or light [16]. The rate of deterioration particularly depends on the chemical composition of the beer, which in turn is determined by the type, quality, and amount of raw materials used in the manufacturing process, as well as by the treatments applied to the wort and beer.

Ensuring non-biological or physical stability, referred to as colloidal stability, is one of the key challenges for brewers considering modifications to the composition of raw materials, such as enzyme treatment or the addition of adjuncts. So far, studies regarding beer colloidal stability have been mainly focused on identifying haze-active compounds originating from malt or adjuncts and evaluating of their concentrations in beer, as a function of varying malting or mashing conditions [3, 4, 7, 11, 12, 15, 18–25]. Various stabilization techniques have also been investigated [5, 8, 9]. The great majority of research has been conducted on a laboratory scale. To the best knowledge of the authors, there are no reports on the effects of full-scale brewing with adjuncts, enzymes, and stabilization agents on the physical stability of beer. The present study fills this gap, by evaluating the impact of various compositions of raw materials as well as wort and beer treatments on beer colloidal stability.

Materials and methods

Materials

The beers were produced under full-scale conditions in an industrial brewery located in Poland. Five variants of high gravity lager beers were analyzed for their content of compounds related to beer colloidal stability. The physical shelf life of the beers was also determined immediately after bottling. The reference sample was an all-malt beer (100% barley malt). Adjunct beers were produced from mixtures of malt, corn grist, and starch syrup in the ratio 60: 20: 20 (corn beer), as well as from malt and unmalted barley in the ratio 51: 49 (barley beers A, B, and C). Aside from the basic sources of carbohydrates, the main differences between the beers were related to the mashing program, the enzyme treatment (type, dose, and time), and stabilization treatment (type of agent) used. The worts were obtained in a brewhouse consisting of a cereal cooker (for corn beer), mash tun, lauter tun (for all-malt beer), mash filter (for the other beers), wort kettle, and whirlpool. All the beers were filtered using a diatomaceous earth applied on the candle filter.

All-malt beer: Mashing program: 48–52 °C (10 min); 63 °C (60 min); 72 °C (40 min); 78 °C (40 min). The mash was supplemented with commercial exogenous protease Brewlyve™ NP 900 (2.1 g/hL) and β-glucanase Filtrase® (8.7 g/hL) solutions. Knock-out volume: 1607 hL of wort. Silica gel (25 g/hL) and PVPP (15 g/hL) were added to the beer stream during filtration as stabilization agents.

Corn beer: Mashing program: 48–52 °C (10 min); 63 °C (60 min); 72 °C (40 min); 78 °C (40 min). The mash was supplemented with commercial exogenous protease Brewlyve™ NP 900 (1.6 g/hL) and β-glucanase Filtrase® (2.9 g/hL) solutions. Knock-out volume: 2176 hL of wort. Silica gel (25 g/hL) and PVPP (15 g/hL) were added to the beer stream during filtration as stabilization agents.

Barley beer (A): Mashing program: 48–52 °C (40 min); 63 °C (60 min); 72 °C (40 min), 78 °C (10 min). The mash and the pitching wort were supplemented with commercial exogenous β-glucanase solutions: Filtrase® (4.5 g/hL) for the mash and Finizym 250 L (6.0 g/hL) for the pitching wort. No exogenous proteases were added. Knock-out volumes: 2349 hL of wort. Silica gel (25 g/hL) and PVPP (15 g/hL) were added to the beer stream during filtration as stabilization agents.

Barley beer (B): Mashing program: 48–52 °C (40 min); 63 °C (60 min); 72 °C (40 min), 78 °C (10 min). The mash was supplemented with commercial exogenous protease

Brewlyve™ NP 900 (4.4 g/hL) and β-glucanase Filtrase® (6.0 g/hL) solutions. The β-glucanase enzyme Finizym 250 L solution was also added to the pitching wort (6.0 g/hL). Knock-out volume: 2352 hL of wort. Silica gel (25 g/hL) and PVPP (15 g/hL) were added to the beer stream during filtration as stabilization agents.

Barley beer (C): Mashing program: an appropriate amount of ground unmalted barley was mashed at 48–52 °C (20 min). After 20 min, part of the ground barley malt was added. Mashing-in proceeded for another 25 min. Simultaneously, the rest of the milled barley malt was mashed in another mash tun at 48–52 °C (10 min). After mixing, mashing was performed at 63 °C (60 min), 72 °C (30 min), and 78 °C (30 min). The mash was supplemented with commercial exogenous protease Brewlyve™ NP 900 (4 g/hL) and β-glucanase Brenn Zyme BGD2L L (5.6 g/hL) solutions. Protease enzyme solution Prolyve PAC 30 L (2 g/hL) and β-glucanase enzyme solution Finizym 250 L (1 g/hL) were also added to the pitching wort. Knock-out volume: 2178 hL of wort. The stabilization agent carrageenan Whirlfloc GCE (3 g/hL) was added to the wort 10 min before the end of boiling. The proline-specific endoprotease Brewers Clarex® (1.3 g/hL) was added to the pitching wort.

The fermentation process was carried out in cylindroconical vessels (seven vessels per variant of beer), with the bottom-fermenting yeast *Saccharomyces pastorianus*. Once the fermentations were completed, the yeasts were harvested, the fermentation vessels were cooled, and the precipitates were collected. Subsequently, the green beers obtained were centrifuged, lagered (conditioned), and filtered. Finally, the beers were bottled and pasteurized. Five variants of bottled beer were submitted for both haze forcing and analytical tests.

Methods

All analyses (except for the analyses of total and hydrophobic polypeptides) were performed according to the protocols published in Analytica EBC [26] or MEBAK (Mitteleuropäische Brautechnische Analysenkommission) [27]. The following methods were used to investigate the content of compounds related to beer colloidal stability: the Kjeldahl method (Analytica EBC 2000) for total nitrogen content; coagulable nitrogen (MEBAK 2002); the Bradford Assay for total and hydrophobic polypeptides according to [28]; spectrophotometry (Analytica EBC 2002) for total polyphenols. All the samples were degassed prior to testing using a lab rotary shaker until all the gas had been released.

The colloidal stability of the beers was determined by a haze forcing test—i.e., by accelerating the development of haze according to Analytica EBC 1963 [17]. The beer was held at 60 °C for 7 days, then cooled to 0 °C for 24 h (one full cycle). The haze in EBC units was measured at an angle of 90°. The number of months of predicted shelf life was calculated according to the formula

$$CS = 3 \cdot \left(n - 1 + \frac{2}{t}\right),$$

where *CS* is colloidal stability (values rounded up/down to the nearest half of the month), *n* is the number of cycles needed to exceed 2.0 EBC units, and *t* is turbidity > 2.0 EBC units.

Reproducibility

All analyses were conducted in at least triplicate. Data were expressed as the mean ± standard deviation. Statistical analysis was performed using STATISTICA 13 (Dell, Round Rock, TX, USA). To investigate the variability between different samples, one-way analysis of variance (ANOVA) followed by the Tukey honest significance test (HSD) was performed at a significance level $\alpha = 0.05$.

Results

We investigated the effects of partial substitution of malt with corn and starch syrup, as well as with unmalted barley, on the physical shelf life (colloidal stability) of lager beers. Various mashing conditions, as well as enzyme and stabilization treatments, were considered to determine whether brewing with unmalted barley can produce similar quality beers in terms of colloidal stability compared to all-malt beer. We considered prolonged protein rest during mashing, the addition of commercial exogenous proteases, and the use of alternatives to conventionally exploited silica gel and PVPP (i.e., proline-specific endoprotease and carrageenan). Finally, the results of predictive shelf-life tests were correlated with the concentrations of beer compounds commonly identified with haze and colloidal instability, such as proteins and polyphenols.

Colloidal stability

Figure 1 shows the results of predictive shelf-life tests for five lager beers. The colloidal stability of the beers was in the range of 4–11 months. Therefore, the estimated time after which visible haze develops during storage was clearly influenced by the raw materials used for beer production.

According to the literature, one of the positive effects on beers of the partial substitution of barley malt with unmalted raw materials may be improved colloidal stability [1, 2]. As shown in Fig. 1, the use of corn grist and starch syrup as up to 40% of the total grist had a positive effect on colloidal stability. The beer showed no tendency for visible haze formation (turbidity level more than 2.0

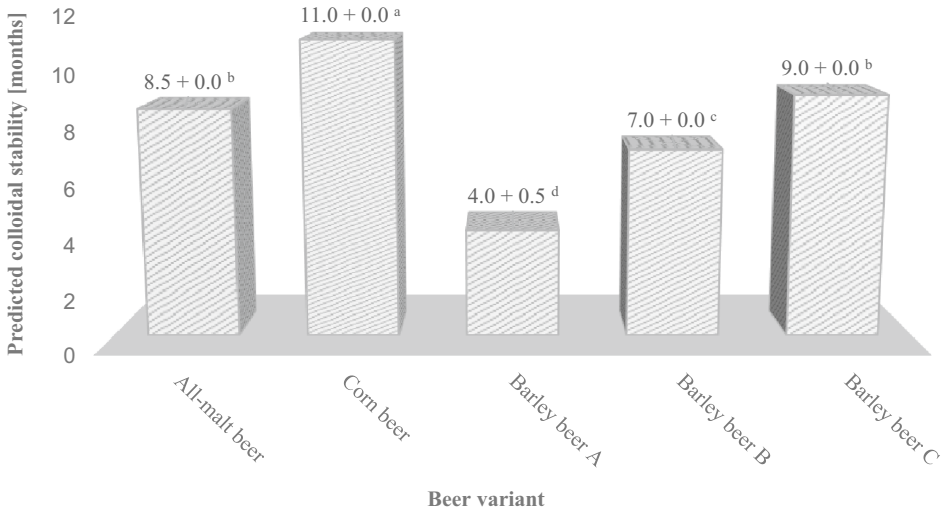

Fig. 1 Predicted colloidal stability of the all-malt and adjunct beers. *Values marked by different letters (**a**–**d**) differ significantly $\alpha = 0.05$

EBC units) for a predicted storage period of 11 months. This was the longest time recorded. The physical shelf life was extended by nearly 30% compared to the all-malt beer (8.5 months). In the case of beer brewed with unmalted barley, the effects were more ambiguous, and were clearly influenced by the enzyme and stabilization treatments. The protease-untreated barley beer A was characterized by the poorest colloidal stability (4 months). Colloidal stability was improved (to 7 months) by the addition of exogenous protease Brewlyve™ NP 900 and a higher dose of β-glucanase Filtrase® during mashing (barley beer B). However, the physical shelf life remained shorter than that of the all-malt beer. Only barley beer C had a longer shelf life (9 months), which presumably resulted from the difference in the stabilization treatment. After treatment with carrageenan and proline-specific endoprotease, the physical shelf life was extended by approximately 5 months in the case of barley beer A and by about 2 months in the case of barley beer B, in comparison to the silica- and PVPP-treated beer. The physical shelf lives of barley beers A and B were extended by a half a month compared to the beer manufactured solely from barley malt (Fig. 1).

Chemical composition of beers

Table 1 shows the differences in the content of total and coagulable nitrogen, total and hydrophobic polypeptides, and total polyphenols in the beers. The concentration of total nitrogen was in the range of 490–1106 mg/L. The concentration of coagulable nitrogen was in the range of 13–31 mg/L. Only barley beer A met the quality standards specified in the literature for total nitrogen, and only beer C met the quality standards for coagulable nitrogen. However, a very weak and statistically insignificant negative association was noted between total nitrogen and colloidal stability ($r = -0.22, n = 5$). In contrast, the coagulable nitrogen content was significantly correlated with colloidal stability ($r = -0.91, n = 5, P < 0.05$). Thus, barley beer A, which was characterized by the poorest physical shelf stability (4 months), exhibited the highest content of coagulable nitrogen. Corn beer showed a slight deficiency of coagulable nitrogen, which presumably contributed to its lower haze-forming potential. The content of hydrophobic polypeptides did not differ substantially depending on the raw materials used in the brewing process. Only a moderate and statistically insignificant negative association was found between the content of hydrophobic polypeptides and colloidal

Table 1 Chemical compositions of the all-malt beer and adjunct beers

Parameter	Literature	All-malt beer	Corn beer	Barley beer A	Barley beer B	Barley beer C
Total nitrogen [mg/L]	700–800 [3]	868 ± 14^{b}	490 ± 14^{d}	747 ± 8^{c}	849 ± 8^{b}	1106 ± 14^{a}
Coagulable nitrogen [mg/L]	15–25 [3]	26 ± 1^{b}	13 ± 2^{d}	31 ± 2^{a}	28 ± 2^{ab}	20 ± 1^{c}
Total polypeptides [mg/L]	–	263 ± 3^{b}	177 ± 3^{e}	249 ± 2^{c}	275 ± 3^{a}	220 ± 1^{d}
Hydrophobic polypeptides [mg/L]	–	51 ± 2^{a}	44 ± 4^{b}	49 ± 1^{ab}	47 ± 2^{ab}	49 ± 1^{ab}
Total polyphenols [mg/L]	50–300 [2, 8]	156 ± 3^{a}	107 ± 1^{e}	121 ± 3^{d}	187 ± 2^{b}	246 ± 1^{a}

*Values marked by different letters (**a**–**e**) on the same line differ significantly $\alpha = 0.05$

stability ($r = -0.47, n = 5$). Lower total nitrogen contents were observed with the Bradford assay than with the Kjeldahl method, which is in accordance with findings reported by Steiner and Back [29]. The Bradford assay is believed to be a more reliable method for the analysis of total proteins in a beer, as it is less prone to interference from non-protein and nitrogen-containing compounds [29]. It may therefore be considered a suitable alternative method for the general investigation of proteins, which may affect many quality attributes of beer, such as a haze-forming potential during storage. A strong but insignificant correlation between beer colloidal stability and total proteins (polypeptides) was observed ($r = -0.67, n = 5$). For instance, the samples with the longest physical shelf life (corn beer (11 months) and barley beer C (9 months)) were characterized by the lowest content of total polypeptides (Table 1).

Given that PVPP treatment leads to a substantial reduction in the concentration of total polyphenols, it was expected that barley beer C would feature a significantly higher content of these compounds. As shown in Table 1, the level of total polyphenols in the beers stabilized with PVPP was in the range of 107–187 mg/L, whereas the enzyme-treated barley beer C contained approximately 246 mg/L of polyphenols. This is in line with findings reported by Lopez and Edens [5]. In our study, no association was found between beer colloidal stability and total polyphenol content ($r = 0.06, n = 5$).

Discussion

A shelf life of 6 months to 1 year is now considered normal for beers produced on an industrial scale [30]. Protease treatment during mashing appears necessary to meet this basic requirement when brewing with up to 49% unmalted barley in the total grist. It is speculated that the addition of proline-specific endoprotease during fermentation can enable further improvement of beer colloidal stability and the production of beers with similar haze-forming potential to all-malt beer. Previous studies established that proteins have a decisive impact on beer colloidal stability [12, 21, 24]. Researchers have also investigated the influence on colloidal stability of the hydrophobicity and molecular weight of proteins [11, 15, 31]. Although high molecular weight polypeptides and in particular hydrophobic polypeptides are primarily associated with the beer head stability, the formation of haze may also be associated with high molecular weight hydrophobic polypeptides originating from the hordein fraction of barley grain [5, 24, 31]. In the initial stage of haze formation, there is interaction between hydrophobic proteins and phenols, followed by the oxidation and polymerization of the resulting complex. This leads to the formation of more hydrophobic proteins of even higher molecular weights and eventually to haze [31].

Given that the Kjeldahl method measures a wide range of low molecular weight nitrogenous compounds (e.g. nucleic acids, amino acids), the Bradford assay appears to be a more useful method of quantifying proteins. No significant correlation was found between beer colloidal stability and the content of hydrophobic polypeptides. This does not necessarily signify that the lower colloidal stability measured for barley beer A could not have stemmed from the higher level of hydrophobic polypeptides, which may have resulted from the substitution of malt with unmalted barley and the concomitant deficit of proteolytic activity. As stated by Siebert and Knudson [32], the Bradford assay is much more sensitive to proteins rich in arginine, histidine, and lysine than to other proteins of similarly high molecular size, such as proline-rich proteins involved in haze formation. It can be speculated, therefore, that brewing with up to 49% unmalted barley and simultaneous abandonment of exogenous protease treatment could lead to higher contents of haze-active proteins and lower beer colloidal stability. On the other hand, supplementing the mash and/or pitching wort with enzymes, especially proline-specific endoprotease, may help extend the physical shelf life of beer, which is presumably related to the lower molecular weight and hydrophobicity of the hordein fraction. The results of coagulable nitrogen analysis seem to reinforce this assumption. According to the literature, coagulable nitrogen is associated with high molecular weight proteins, which precipitate in large part during wort boiling and are subsequently removed during whirlpooling [33].

Concentrations of coagulable nitrogen in the range of 15–25 mg/L (or, as recently suggested, 20–30 mg/L) are considered to positively affect both head retention and mouthfeel, as well as beer colloidal stability [3, 33, 34]. Steiner et al. [3] compared the quality attributes of beers manufactured solely from malt and unmalted barley. They reported considerably higher coagulable nitrogen content in beer made from barley raw material (21 mg/L) than in all-malt beer (17 mg/L). This was explained by the lack of proteolysis. Proteolysis occurs during the malting process and entails the initial degradation of high molecular weight proteins. The tendency for coagulable nitrogen content in wort to rise with increasing proportions of unmalted barley in the grist has also been reported by Kunz et al. [4]. These findings may explain the excessive coagulable nitrogen content we measured in barley beer A (Table 1), and its short physical shelf life (4 months) compared to all-malt beer (8.5 months), barley beer B (7 months), and barley beer C (9 months) (Fig. 1).

Barley beer C treated with carrageenan and enzymes was stable for a significantly longer storage period than barley beers A and B (Fig. 1). It was also characterized by a

significantly elevated content of total polyphenols (Table 1). Given the high capital costs of PVPP regeneration and the inevitable lowering of natural antioxidant potential [5], it is therefore advisable for beer brewed using up to 49% unmalted barley in the total grist to be stabilized by proline-specific endoprotease treatment, preferably in combination with carrageenan.

The longest physical shelf life observed for corn beer was presumably the effect of diluting the wort and beer with coagulable nitrogen, polypeptides, and polyphenols (Table 1). On one hand, corn grist contains fewer nitrogen compounds, and starch syrups contain none whatsoever. On the other hand, proteins present in corn grist are not extracted during mashing to the same extent as proteins in barley malt, since they do not undergo thorough hydrolysis in the malting process [20]. Therefore, when increasing the share of corn grist and starch syrups, commercial exogenous proteases should be used to provide sufficient free amino nitrogen levels for yeast nutrition.

Conclusions

The results presented in this study provide valuable insight into the haze-forming potential of lager beers brewed with various raw materials and process aids. We assessed the impact of partial substitution of malt with corn grist and sugar syrup or with unmalted barley. We also examined how the addition of exogenous proteases and carrageenan affected colloidal stability. Predicting haze formation during storage based on total nitrogen content (by the Kjeldahl method) and polyphenol content (by spectrophotometry) in the final beer after bottling was found to be difficult. However, measuring and adjusting the content of coagulable nitrogen offers a powerful way to estimate and reduce haze-forming potential. The colloidal stability of beer produced from unmalted barley can be improved by supplementing the mash/wort and/or pitching wort with exogenous proteases and carrageenan. The beers with added corn grist and starch syrup also showed significantly enhanced physical shelf life. The results of this study show that careful selection of raw materials and process aids can significantly improve the colloidal stability of beers produced on an industrial scale.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not contain any experiments with human or animal subjects.

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