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Assay for detecting technical proteolytic enzymes in beer

The chemical-physical stability is an important characteristic of filtered beer. Since proteins are a main cause of haze in beer, different methods in praxis application to reduce this haze formation have been developed such as an adsorption on silica gel or bentonite as well as an enzymatic breakdown. Since proteolytic enzymes can have a very selective activity on their substrate and are easy to handle, this type of stabilization is often used outside the German purity law. However, due to high inactivation temperatures and an application after boiling process, activities of these enzymes can still be present in the bottled beer. To detect potential rest activities, the aim of this publication was to develop a sensitive and reproducible method for the detection of the proteolytic enzyme activity in beer. Due to the specific activity of these enzymes on proline containing proteins, a proline rich substrate (StabiProlin®) was dosed into the beers and the residual concentration of gliadin (substrate) was determined after 24 hours of incubation. Blank samples resulted in no significant ($P > 0.05$) decrease in gliadin concentration, whereas the content of gliadin in beer containing proteolytic enzymes was significantly ($P < 0.05$) reduced over time. Despite pasteurization up to 1000 PE of the enzyme-inoculated beer, a high breakdown of gliadin (20 – 100 %) could be determined. This was also confirmed in 11 practice beer samples (20 – 75 %). Purity law brewed beers showed no decrease in gliadin concentration. Thus, application of this method can be recommended to optimize beer stabilization using proteolytic enzymes, to select suitable enzyme preparations and especially to adjust the dosage amount of stabilizing enzymes.

Descriptors: beer stabilizer, residual activity, beer haze, filtration

1 Introduction:

Consumers expect, not only in lager beer, a clear product without flakes or haze until the best before date [8, 15]. In order to achieve this characteristics, various processes have been developed in practice to remove or selectively reduce potential haze inducing substances from beer. The main haze sources in beer are proteins and polyphenols. Particularly proline-rich proteins greater 10 kDa from the malt were identified as haze active. In contrast, the polyphenols from hops have been identified to cause severe haze formation, even if their share are only 20 – 30 % of the total polyphenol content in beer. Proteins and polyphenols can interact via hydrogen bonds, resulting in an increase in particle size and ultimately in the formation of haze particles [18, 17, 19].

In order to prevent this haze formation by proteins and polyphenols, it is therefore sufficient to selectively reduce only one of these haze causing substance groups [14–16]. Protein stabilization can be performed with different agents through simple sedimentation or continuous contact methods [2]. Common agents for beer stabilization on proteins are silica gels (adsorption), tannins (precipitation) or proteolytic enzymes (degradation). For the application of these stabilizing agents national and international regulations must be observed. Within the German purity law, the application of these substances is restricted depending on the respective reaction mechanism [1]. Only agents that remove the haze inducing substances via adsorption and which are 100 % removable are permitted in German beer production [3]. Therefore, this country-specific regulation does not allow the use of enzymes, while the use of technical enzymes in the brewing process is widespread in the rest of the world. Enzymes for beer stabilization have the advantage of a simple dosage, no necessary powder handling with the required system technology and a small influence on the beer color [1]. Two types of enzymes are common in stabilization: papain (a mixture of proteolytic enzymes from papaya) or proline-specific peptidases [9, 11]. The treatment with papain was already described in 1911 [4]. Although this application has been popular for many years, the activity of this enzyme mixture is not specific for haze-causing proteins and is therefore susceptible to foam stability [9]. In comparison, the other type of enzyme is specific to haze-causing polypeptides rich in proline and therefore has little impact on the beer foam. Even if both types of enzymes reduce the formation of beer haze reliable, there are problems with the inactivation of these enzymes. The addition of technical enzymes to the brewing process after the boiling step in the brewhouse bears the risk of remaining enzyme activity in finished beer. Pasteurization does not inactivate all added technical enzymes. The regulatory status of the enzyme activity in the finished beer remains unclear. The Guidance Document on Criteria for Categorization

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of Food Enzymes of the EU Commission concludes that the use of such enzymes needs to be declared if the „Food enzyme performs or can perform a technological function in the food as marketed or as prepared by the consumer“ [5]. That is leaving space for debate and dissent. This article is not meant to make regulatory interpretations or legal claims in that respect. However, consumers and brewers are interested to understand whether the presence of added technical enzymes can be proven in the final product. Suitable test methods exist for the proof of β-glucanase and amylase enzymes. A practical way is based on the principle to measure the decrease of a specific substrate, but due to the large distribution of proteins and the associated specific proteases, this is not easy when measuring the activity of these enzymes in beer. For this reason, the aim of this publication was to test a selective substrate and develop a method for the detection of proteolytic enzyme activity in beer. This method should be easy to perform in brewery laboratories. A new approach was tested a prolin rich extract that is used to mimic protein-polyphenol interactions in beer and to predict haze-forming patterns in beer at lab scale. In addition, a validation with practice samples was performed to demonstrate applicability in the brewing industry.

2 Materials and methods

2.1 Materials

To validate the reproducibility of the test and to determine the error value as well as screening different beer samples with the developed method a set of various beers from all over the world were used (including gluten-free beer) as test material. Beers that were produced within the German purity law (no exogenous enzyme permitted) were purchased as blank samples (reference). These were used to test various enzyme concentrations and products as well as the pasteurization and to determine the error in the methodology. In addition, differently stabilized beers were purchased from Finland, Denmark, Belgium and Mexico for which the use of enzymes was declared or suspected.

The proline rich substrate StabiProlin® (Stabifix Brauerei-Technik GmbH & Co. KG, Gräfelfing, Germany) was used as the substrate for the test of proteolytic enzyme activity. The ethanol extract contains 55 % nitrogen, 85 % of which is gliadin (3400 ng/mL). The gliadin content in the samples was quantified using the ELISA assay RIDASCREEN® Gliadin competitive (r-biopharm, Darmstadt, Germany).

Three proteolytic stabilizing enzymes were tested to validate the method. These included papain (Merck KGaA, Darmstadt, Germany) and 2 endoproteases (Proline-specific endoprotease - WLE4000, Clarity Ferm, White Labs, San Diego, USA; Brewers Clarex®, DSM Food Specialties). PVPP (Divergan F, BASF, Germany) was used to remove polyphenols from the beer samples. Pleated filters (MN 514 ½, Macherey-Nagel, Germany) are used for filtration.

2.2 Sample preparation and method procedure

Before the proline substrate could be added to the beer, haze-active polyphenols had to be removed. This had to be done in order to prevent the formation of haze complexes after the dosage of the proline substrate and to avoid false-positive results. Thus, 1 g/L PVPP (Divergan F, BASF SE, Ludwigshafen Germany) was added into beer and stirred for 10 min. This corresponds to twice the maximum prescribed dosage for stabilizing beer in order to ensure a sufficient reduction in polyphenols [1]. Afterwards PVPP was filtered off through a pleated filter.

To determine the proteolytic enzyme activity, the stabilized beer was divided into 100 ml sample bottles and 1 ml proline rich substrate was added into the beer. The blank sample was taken and frozen immediately. All approaches were carried out at least in duplicate. For the experiments with enzyme incubation, the dosage of the enzymes was performed before incubation. In order to exclude a reaction by the stabilizing enzyme may present in beer, purity law produced beers were used for these experiments as blank samples. The practice samples were not treated further for the analysis (no enzyme addition). The reaction for enzymatic degradation was carried out at 35 °C for 6, 24 or 48 hours. Samples were taken again after the incubation.

Afterwards, the gliadin content was measured in all samples using the RIDASCREEN Gliadin competitive (r-biopharm, Darmstadt, Germany) according to instructions of the manufacturer in duplicate. The concentration was examined using RidaSoft Win.NET (Version 1.84, r-biopharm, Darmstadt, Germany).

2.3 Validation of the functionality of the methodology

To proof the methodology, different blank samples and beers with enzyme dosage were examined. For this purpose, the three enzymes (two proteases and one papain) were dosed into a beer brewed within the German Purity Law. The used concentrations were calculated using dosage instructions from the manufacturers. Four concentrations 8 (for haze stabilization purposes), 9.6 (for gluten reduction purposes with less than 20 % wheat malt), 12.8 (for dry-hopped beer) and 19.6 µl/100 ml (for gluten reduction purposes with more than 20 % wheat malt) were investigated to validate the methodology.

Table 1 Temperature and incubation time of the pasteurisation trials – procedure for inactivation of enzymes

Pasteurisation units [PU]	Incubation time at maximum temperature [min]	Maximum temperature [°C]
0	-	-
10	2	64.8
50	2	69.7
100	2	71.8
250	2	74.6
500	2	76.7
1000	2	78.7
1500	2	80.0
3000	2	82.1
10000	2	85.7

2.4 Pasteurisation trials

Besides the validation of the functionality of the method a next step should be a test of inactivation of the stabilizing enzymed. For this reason, protease 1 was inoculated in beer brewed within the German purity law at a concentration of 8 µl/100 ml and pasteurized using table 1. At the end of the heat treatment, a sample was taken and tested for the content of residual gliadin as previously described. In addition, the other two enzymes were randomly checked at 3 temperatures (10, 100 and 1000 PU).

2.5 Statistics

Statistical analyses to determine averages and standard deviations as well as ANOVA were carried out using OriginPro 2020 (version 9.7.0.188, OriginLab Cooperation, Northampton, USA).

3 Results

The aim of this publication was to establish a simple method for the detection of possible residual activities caused by proteolytic stabilizing enzymes. In order to implement the method as simply as possible, established analyzing methods were used. Since proline-containing proteins are particularly known to cause haze, the concentration of haze active proteins was determined using the ELISA assay RIDASCREEN® Gliadin competitive. The activity of the enzymes was demonstrated with a special proline standard which was added to the beer in order to increase the haze active protein content. In order to test the functional principle of the method and to check the stability of the standard, a blank sample was first inoculated with the proline rich extract and then incubated for 48 h at 35 °C. The results of the blank sample are shown in figure 1. No significant change ($P > 0.05$) in the gliadin concentration of the blank sample could be detected during the 48 h sampling. Thus, verification of the stability of the gliadin substrate in beer without proteolytic enzyme activity could be demonstrated. In comparison, the beer was additionally inoculated with an endoprotease (enzyme 1) and the concentration of gliadin was examined after 6, 24 and 48 h. Figure 1 shows the decrease in gliadin concentration due to the impact of the proteolytic enzyme over the sampling period. The greatest decrease could be examined after 6 h. The difference in gliadin between 6 h and 24 h was very small. After an incubation time of 48 h, further degradation in gliadin content was detected.

Gliadin content decreased significantly between 0 h and 6 h ($P < 0.05$) as well as 24 h and 48 h ($P < 0.05$), but not between 6 h and 24 h ($P > 0.05$). Similar results were found in all tests with enzyme dosage (data not shown, n = 8×2). The average variance of the analysis method was found to be 6.6 % for the blank samples and 6.4 % for the enzyme spiked trials.

This shows that the methodology is functional. Since the method is intended to provide information quickly, sampling was omitted after 24 h. A complete measurement including the determination of the gliadin concentration was not possible within one working day. For this reason, a measurement after 24 h was the fastest option. The experiments showed that a stable measurement was possible after 24 h. In addition, there should be no complete degradation of the substrate in order to detect a residual concentration and thus determine a difference between different enzyme preparations. In addition, it cannot be assumed that the enzyme activity in the practice samples is so high that sufficient gliadin degradation will be achieved after 6 h.

To check the selectivity of the assay for different enzyme activity, four enzyme concentrations were tested taken from the manufacturer's dosage information. In order to be able to assess the impact, further spiking trials were carried out with beer brewed within German purity law. Figure 2 shows the concentration of gliadin as a function of the enzyme concentration of protease 1.

The results show that the higher the proteolytic enzyme concentration, the higher the decrease in gliadin after 6 h of incubation. In addition, no significant difference ($P > 0.05$) could be seen after 24 h in the three high enzyme concentrations (9.6, 12.8 and

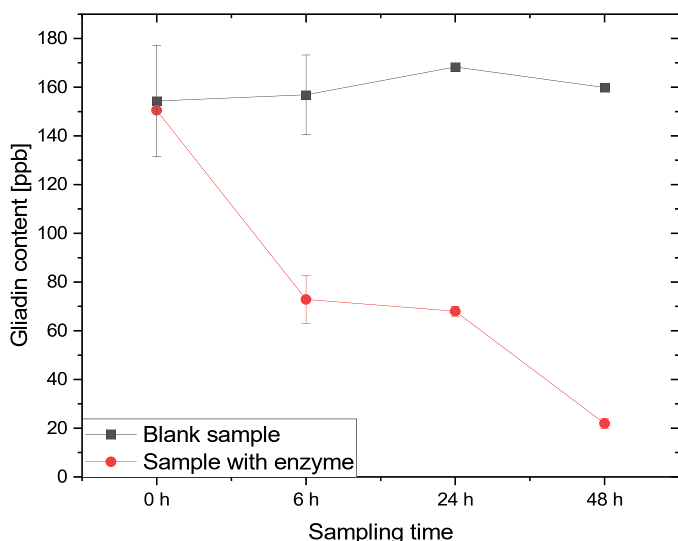

Fig. 1 Gliadin concentrations (n = 2×2) in a blank sample and inoculated sample with enzyme depending on the time of measurement

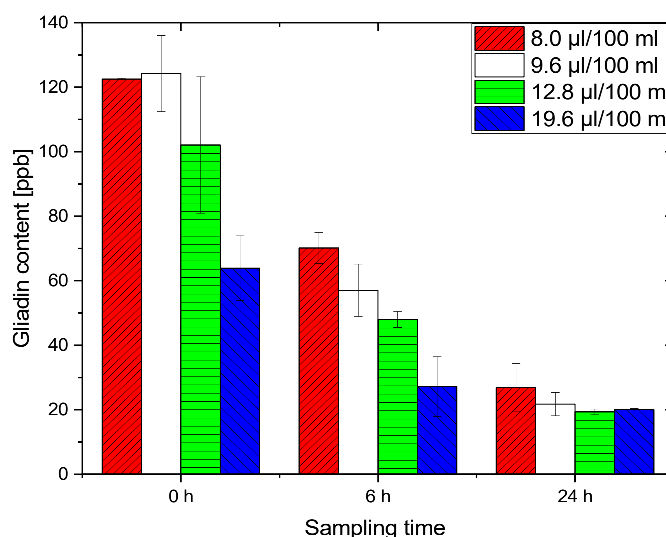

Fig. 2 Gliadin concentration (n = 2×2) depending on the enzyme concentration of the endo-protease 1 and sampling time

Fig. 3 Impact of enzyme concentration on gliadin content (n = 2×2) in dependence to incubation time

19.6 µl/100 ml). It was noticeable that the sample at beginning of the trials showed significant differences ($P < 0.05$) in the gliadin concentration between the three lower and the highest enzyme concentration. Since the blank sample was taken after addition of the enzyme, the activity of the enzyme could have been sufficient to reduce the difference in gliadin until complete freezing. Looking at the data compared to the concentration of the blank sample at 0 h (see Fig. 3a), it is seen that the gliadin concentration steadily decreases. The concentration of the enzymes seems to play a subordinate role at protease 1. This could indicate that the dosage of the enzyme was too high for the existing haze-relevant protein content. Comparable results could be observed with protease 2 (see Fig. 3b). Enzyme 3 (papain) had the lowest reduction of gliadin over time. An impact of the enzyme content could be measured, whereby the two mean concentrations (9.6 and 12.8 µl/100 ml) resulted in a comparable degradation (see Fig. 3c). This is also an indication of the excessive dosage of protease 1 and 2 (enzyme 1 and 2). Since the manufacturer's instructions were observed for the dosage, it can be assumed that comparable concentrations are used in practice beer samples.

Nevertheless, in all three tested proteolytic enzymes highest enzyme concentration resulted in highest decrease in gliadin content. Analysis of variance using ANOVA showed that 8 and 9.6 µl/100 ml were significant ($P < 0.05$) different to 19.6 µl/100 ml at 6 h examining enzyme 1. No significant ($P > 0.05$) differences at other concentrations or at 24 h could be observed. Enzyme 2 resulted in significant ($P < 0.05$) differences between 8 µl/100 ml to all other enzyme concentration at 6 h, but not at 24 h. The third examined enzyme resulted in significant ($P < 0.05$) difference in all low enzyme concentrations to 19.6 µl/100 ml at 6 h. With regard to sampling time no significant ($P > 0.05$) differences at 24 h could be determined.

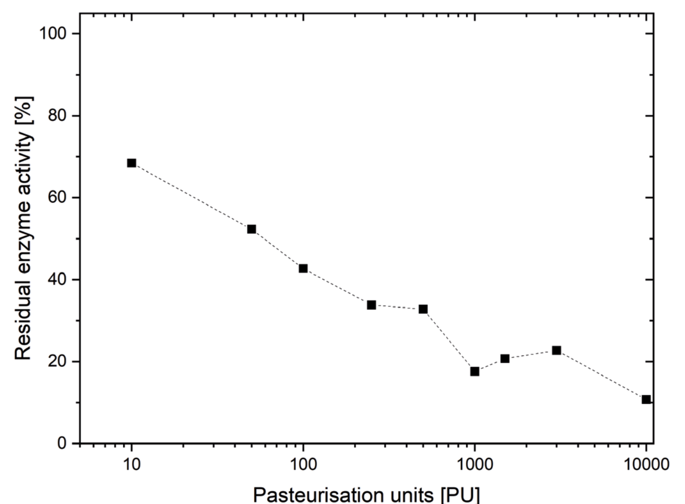

Fig. 4 Detection of residual activity of enzyme 1 in % (n = 2×2) in dependence to the applied pasteurization units

Table 2 Detection of residual activity of the three enzyent in % (n = 2×2) in dependence to the applied pasteurization units

Pasteurisation units [PU]	Protease 1 [%]	Protease 2 [%]	Papain [%]
10	68.4	1.5	17.5
100	42.7	0.1	12.5
1000	17.6	– 19.8	– 17.9

In addition to the enzyme spiking trials, the impact of pasteurization on residual enzyme activity should be investigated. This was necessary for the subsequent determination of practice samples, since pasteurization is the last process step in the brewery, where technical enzymes could be inactivated. If the enzymes were already inactivated at low temperatures, detection in the manner described would not be possible. Thus, pasteurization between 10 and 10,000 PU was tested in order to determine the inactivation temperature of protease 1 (enzyme 1). The results of the pasteurization trials are shown in figure 4.

It could be shown that an increasing pasteurization treatment resulted in a lower activity of the proteolytic enzyme. Above 1000 PU a residual activity of less than 20 % could be measured, which continued to decrease up to 10,000 PU (~10 %). Taking into account the determined method error, an inactivation greater than 1000 PU can be assumed. In comparison, the other two enzymes had a higher inactivation at 10 respectively 100 PU (see Table 2). The results show that the method can examine clear differences in activation between the used enzymes. In consequence, protease 1 is very temperature stable. In practice, with common pasteurization units between 20 – 50 PU, this enzyme would not be inactivated and the enzyme activity would still be measurable in final beer. In comparison, enzymes 2 and 3 have only a low residual activity after pasteurization. The high thermal influence at 1000 PU resulted in negative activities. This could have resulted in the release of bound proteins, which may have influenced the concentration of measurable gliadins. The results shows the need to establish a method that is able to detect the residual proteolytic enzyme activity after beer stabilization to optimize the selection and dosage of the enzymes.

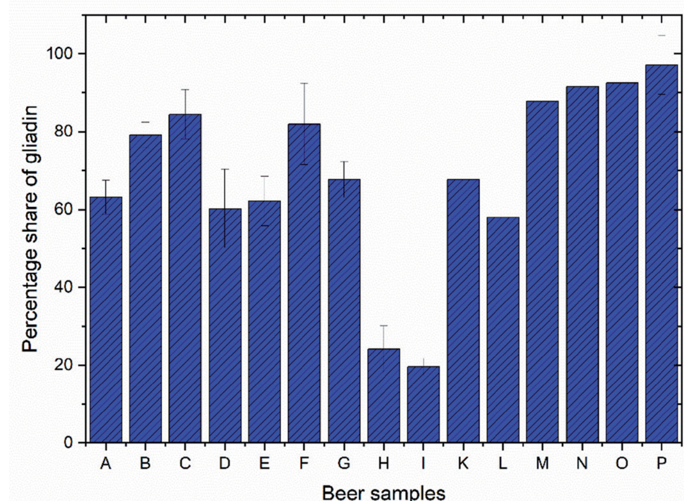

Fig. 5 Percentage share of gliadin after 24 h incubation time in comparison to blank sample of 15 beer samples – A–F – non-purity law brewed (without declaration of enzymes), G–I – glutenfree beer (endoprotease), K-L beer with papain stabilisation, M-P purity law brewed beer

Since the measurement has proven to be functional on the basis of the various tests, practice beer samples were examined with regard to remaining proteolytic enyzme activity. In addition to beer brewed within the German purity law (blank samples), various beers were purchased from other European countries. These samples also included gluten-free beers that are advertised with the use of a protease. Figure 5 shows the remaining percentage of gliadin after an incubation at 35 °C for 24 h. To check the methodology, again beers brewed within the German purity law were examined as reference samples. A decrease of less than 8 % (n = 4, min: 87.9 %, max: 97.1 %) in gliadin content could be observed in these beer samples. In comparison, the non-purity law brewed beers had an average of 60.7 % remaining gliadin (n = 11) and thus a decrease by 39.3 %. The highest decrease could be observed when investigating gluten free beer with an average of 37 % remaining gliadin after 24 h incubation (n = 3, min: 19.5 %, max: 67.7 %). By most of the investigated samples it was not labeled which proteolytic enzymes were used to stabilize the beer and break down gluten. The use of the enzyme type (papain) was only declared on the label for 2 samples. These papain stabilized beer had an average remaining gliadin content of 62.9 % (n = 2, min: 58.1 %, max: 67.7 %). The remaining beers had a decrease to an average of 71.9 % (n = 6, min: 60.2 %, max: 84.5 %).

The statistical differentiation using ANOVA shows that gluten-free beers (H–I) and the non-purity law brewed beer (A–F) were significantly different ($P < 0.05$). There was no significant difference ($P > 0.05$) between gluten-free and papain-stabilized beers. All samples with added enzymes for stabilization application (suspected or declared) had a significant difference ($P < 0.05$) to the German purity law beer. The method allows a clear distinction between beers which are produced within the German purity law and those to which enzymes were added to stabilize proteins or to break down gluten in order to produce gluten-free beers. In addition, it is possible to detect residues from high enzyme use which are used for the production of gluten-free beers. However, it is not possible to differentiate between the enzymes used. This shows that, despite pasteurization, permanent enzyme activity was detectable in the practical samples made with exogenous enzymes.

4 Discussion

The aim of this publication was to develop a simple and rapid method to determine the activity of remaining proteolytic enzymes in final beer. Various methods have been established over the years to determine the activity of papain or other proteolytic enzymes which cannot be used for practical applications or where the substrates are no longer available. A first approach to determine papain in beer was shown by *Scriban* and *Stienne* [13]. Papain activity was measured by the rate of release of remazolbrilliant blue from insoluble hide-powder covalently labelled with the dye. The disadvantage of this method was that the measurement was made at pH 4.0 and not at beer pH, which could influence the

enzyme activity. A further limitation of this method was a possible release of the dye by non-enzymatic components in beer. Due to the dye reaction, it was not possible to investigate dark beers (over 40 EBC). In addition, no practice samples but only enzyme spiked samples were examined in the publication [13]. In contrast, Hebert et al. [7] made electrophoresis analyzes of various enzymes, but not directly in the product beer. Aim of their work was an evaluation of different enzymes for the use of beer stabilization. In another approach, denatured ^{125}I-labelled human serum albumin (^{125}I-HSA) was used as a substrate to demonstrate the proteolytic activity in beer [6]. The reaction was proceeded at pH 6.0 and 40 °C. This highly sensitive method for the assay of chillproofing enzymes of unknown origin in beer was functional, but toxic reagents (formaldehyde) had to be used to prepare the standard. In addition, ^{125}I-HSA is not readily available and expensive to purchase. Thus, the application and measurement in routine brewery laboratories is not easily applicable. In 2005, *Lopez* and *Edens* [10] described the use of a gliadin substrate as model substance to determine proteolytic enzyme activity in beer. However, no practice samples were examined, rather the use of a specific enzyme for beer stabilization was demonstrated. A similar approach of the gliadin substrate was used in this publication. With the help of the used proline rich substrate (StabiProlin®) and various stabilizing enzymes, the functionality of the method in beer was demonstrated. As can be seen from the literature research, comparable easy methods are not available.

Although the method is simple to perform, there are several sources of error that must be considered in the measurement. Decreases of up to 10 % in the gliadin content occurred in the blank samples. A slight error would be conceivable due to an interaction of the added proteins to remaining polyphenols and subsequent sedimentation of the proline rich substrate. To minimize this error, a previous PVPP precipitation of haze-active polyphenols was carried out. The concentration of PVPP was chosen so high that extensive precipitation of haze-active polyphenols could be guaranteed. Another source of error may be the measurement of the gliadin content using ELISA. A maximum decrease of 12 % (mean: 8 %) across all blank samples could be determined. Nevertheless, both the spiking trials and the practice samples had significant higher reductions in gliadin content than the blank samples. The functionality of the method with typical stabilizing enzymes could be demonstrated. Different incubation times (6, 24, 48 h) were checked, with 24 h being determined to be functional for the test procedure. Depending on the enzymes investigated, differences in the gliadin concentration could be measured after 6 h but not after 24 h. Since it was not the aim of the method to differentiate enzyme concentrations, 24 h was chosen as the incubation time. This long incubation time was also necessary because the activity in practice beer samples could be damaged by pasteurization that may have been used. The temperature stability of the common stabilizing enzymes could be proven by means of spiking trials. A permanent enzyme activity due to the high enzyme stability is known for papain (34 %) or protease (19.4 %) with a pasteurization at 60 °C for 20 min (25 PU) [12]. This permanent enzyme activity could also be determined in various practical beer samples. The highest decrease in gliadin could be found in gluten free beers, which means that the enzyme activity was highest in these samples. Purity law brewed samples, however, had as expected almost no activity. The examination of the practical samples showed that there are great differences in the permanent enzyme activity of proteolytic enzymes on the beer products in market. Only a few of the samples clearly labeled the use of enzymes for stabilization.

5 Conclusions

A new assay for detection of proteolytic enzyme activity in final beer has been developed. It could be shown that the used proline rich substrate (StabiProlin®) was stable, low in purchase and usable for the application. Since the method should be applied in brewing laboratory for routine operation, a simple methodology of sample preparation (separation polyphenols) was developed. Analysis time sums up to 20 min sample preparation, 24 h incubation time and 3 h measurement time for the gliadin ELISA assay. Thus, optimizations in beer stabilization can be carried out rapid and easily.

Blank samples (reference samples) that were brewed within the German purity law (no exogenous enzymes) had slight changes (max 12 %) in gliadin content after 24 h incubation. Thus, decrease by less than 12 % can be considered as error value, meaning no proteolytic enzyme activity. In contrast, permanent enzyme activity could not only be measured in prepared samples inoculated with enzyme, but also in the practice samples. Inactivation experiments were carried out in order to check the temperature stability of the enzyme preparations examined. A pasteurization of beer samples did not result in an inactivation of all proteolytic enzymes. One of the enzymes examined only resulted in an inactivation of 80 % at 1000 PU. These high pasteurization units, however, are not practical and are not achieved in beer production, since the product would be irreparably damaged in terms of flavor and color. Nevertheless, there were large differences between the tested proteolytic enzymes. This was particularly evident with the tested enzymes protease 2 and papain. These enzymes could be inactivated more quickly and would therefore be more suitable for use as a stabilizing enzyme after the boiling step in the brewhouse. Finally, the methodology was tested and validated on various practice samples. In some of the samples, enzyme activity was permanent despite pasteurization. In summary, the functionality of the method could be confirmed and validated on the basis of the various results. The method is suitable for optimizing the stabilization process of beer using proteolytic enzymes, selecting a suitable type of enzyme and optimizing inactivation by pasteurization.

6 References

1. Annemüller, G. and Manger, H. J.: Gärung und Reifung des Bieres-Grundlagen, Technologie, Anlagentechnik, VLB Fachbücher, Berlin, 2009, Vol. 1.
2. Annemüller, G. and Manger, H. J.: Klärung und Stabilisierung des Bieres, Grundlagen – Technologie – Anlagentechnik – Qualitätsmanagement, Verlag der VLB Berlin, 2011.
3. Bundesamt für Justiz: Verordnung zur Durchführung des Vorläufigen Biergesetzes (BierStDB) 2000.
4. Esnault, E.: Beer stabilization with papain, Brewer’s Guardian, **124** (1995), pp. 47-49.

5. European Commission: Guidance Document On Criteria for Categorisation of Food Enzymes, 24 February 2014.
6. Fukal, L.; Rauch, P. and Kás, J.: A very sensitive method for assaying proteolytic activities in beer, Journal of the Institute of Brewing, **89** (1983), no. 4, pp. 257-259.
7. Hebert, J. P.; Scriban, R. and Strobbel, B.: Analytical Study of Proteolytic Enzymes for Beer Stabilization, Journal of the American Society of Brewing Chemists, **36** (1978), no. 1, pp. 31-38.
8. Kupetz, M.; Frankerl, M.; Gastl, M. and Becker, T.: Rapid method to assess the colloidal haze stability of beer, BrewingScience, **72** (2019), no. 7/8, pp. 142-147.
9. Leiper, K. A. and Miedl, M.: Colloidal stability of beer In: Bamforth, C. W. (ed.): Handbook of Alcoholic Berverages Series – Beer a Quality Perspective, Academic Press, London, 2009.
10. Lopez, M. and Edens, L.: Effective Prevention of Chill-Haze in Beer Using an AcidProline-Specific Endoprotease from Aspergillus niger, Journal of Agricultural and Food Chemistry, **53** (2005), no. 20, pp. 7944-7949.
11. Mastanjevic, K.; Krstanovic, V.; Lukinac, J.; Jukic, M.; Vulin, Z. and Mastanjevic, K.: Beer – The Importance of Colloidal Stability (Non-Biological Haze), Fermentation, **4** (2018), pp. 1-12.
12. Nelson, G. and Young, T. W.: Yeast extracellular proteolytic enzymes for chill-proofing beer, Journal of the Institute of Brewing, **92** (1986), no. 6, pp. 599-603.
13. Scriban, R. and Stienne, M.: Detection and quantitative determination of papain in beer, Journal of the Institute of Brewing, **76** (1970), no. 3, pp. 243-244.
14. Siebert, K. J., Lynn, P.Y.: The Effect of Beer pH on Colloidal Stabilization with Adsorbents, J. Am. Soc. Brew. Chem., **65** (2007), no. 1, pp. 52-58.
15. Siebert, K. J.; Troukhanova, N. V. and Lynn, P. Y.: Nature of polyphenol-protein interactions, Journal of Agricultural and Food Chemistry, **44** (1996), no. 1, pp. 80-85.
16. Siebert, K. J. C., A.; Lynn, P.Y.: Formation of Protein-Polyphenol Haze in Beverages, Journal of Agricultural and Food Chemistry, 44 (1996), no. 8, pp. 1997-2005.
17. Steiner, E., Arendt, E.K., Gastl, M., Becker, T.: Influence of malting parameters on the haze formation of beer after filtration, Journal of European Food Research and Technology, **233** (2011), no. 4, pp. 587-597.
18. Steiner, E., Auer, A., Becker, T., Gastl, M.: Comparison of beer quality attributes between beer brewed with 100 % barley malt and 100 % barley raw material, Wiley Online Libary (2011).
19. Steiner, E.; Becker, T. and Gastl, M.: Turbidity and Haze Formation in Beer – Insights and Overview, Journal of Institute of Brewing, **116** (2010), no. 4, pp. 360-368.

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