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The Influence of Fermentation-Control on the Colloidal Stability and the Reducing Power of the Resulting Bottom Fermented Beers

The focal point of this work was to evaluate variations in fermentation-control, which included different fermentation temperatures and different pitching rates, the usage of yeast from various numbers of generations as well as the application of pressure fermentation. Of particular interest was the monitoring of haze relevant polyphenols and proteins as well as the detection of antioxidative capacity, which was differentiated between fast reducing substances and total reducing power. The force tests showed reproducible lower formation of colloidal haze in the beers resulting from fermentation at lower temperatures and from decreasing numbers of yeast generations. Also lower pitching rates and pressure fermentation seem to improve colloidal stability. The best reducing power, which does not influence colloidal stability in either case, resulted from cold fermentation and higher pitching rates. It has been shown that variations in the field of fermentation performance may have an impact on the colloidal stability. Thus optimising the fermentation performance and pitching technology can be regarded as one further step to improve colloidal stability in a technological way.

Descriptors: Colloidal stability, haze, reducing power, fermentation, yeast

1 Introduction

Preservation of colloidal stability in bottom fermented and filtered beers has emerged as one of the biggest challenges breweries have to meet in the current beer markets, which exhibit an ever increasing tendency to globalisation combined with rising consumer-expectancy to the clarity and quality of beer.

Main haze forming substances in the bottled beer are polyphenols (first of all flavan-3-ols) and proteins. However, also polysaccharides, minerals and metal ions can be detected in haze [1,2,3,4,5,6,7]. Thus colloidal stability mainly depends on the composition of the raw materials malt, hops and brewing water. In addition to a precise selection of raw materials one further way to improve stability is the usage of stabilisation agents. The latter method is quite effective but results in a loss of potentially physiologically active beer components e.g. the polyphenols, to say nothing of the increased costs for stabilisation. One focal point of current research is therefore the "stabilisation in a technological way" by optimizing the brewing process with regard to lower haze formation.

Knowledge about the influence of fermentation control on the colloidal stability and the performance of haze relevant substances during fermentation is still quite low. It has been reported that the concentration of haze sensitive polyphenols decreases during fermentation, most likely caused by the bonding to proteins in the yeast-cell wall. The content of haze relevant substances then increases again at longer storage periods [8,9]. In contrast Bellmer has not found a considerable decrease of flavan-3-ols during fermentation but during storage [10]. Up to now no experiments have been conducted to get more detailed information on the effect of variations in fermentation-control on colloidal stability.

The aim of this work was to evaluate the influence of fermentation-control on the colloidal stability and the reducing power of the resulting beers. In this regard, the polyphenolic spectrum of wort and beer has been measured by HPLC. Further investigation scopes were the effects on the reducing power, detected by electrochemical methods, as well as on the concentrations of haze sensitive proteins in the beer.

2 Materials and methods

Bitter wort (for Pilsner- and Lager beer) was used in these tests: Original gravity 11.3–12.0 g/100 g, final attenuation degree 80–86 %, pH 4.8–5.1. In each sample of one test-batch the same wort was used. Wort was fermented in 20 l fermentation tanks after 10 min. aeration with a sinter candle under pressure (final oxygen content 8–9 mg/l). Yeast strain: W 34/78; pitching rate ~15 Mio cells/ml (if not indicated otherwise), fermentation temperature differed concerning the approach (see below); at attenuation degree of ~78 %: cooling of the green beer to 4 °C for 24 h and cropping of the yeast; maturation for 1 week at 4 °C, removal of the spent yeast; storage for 2 weeks at 0 °C; beer filtration with a combined kieselguhr/sheet filter (sheets 0.6 µm); Bottling and capping in standard 0.5 l NRW-bottles.

Differentiation of the experiments:

A) Fermentation Temperature: 6 tests; each test included the comparison of 3 different fermentation temperatures. 3 tests were conducted to compare 9 °C, 11 °C and 13 °C-fermentation (approach 1), 3 other tests compared 9 °C, 12 °C and 15 °C-fermentation (approach 2).

B) Pitching rate: Comparison of pitching with 15 Mio and 30 Mio cells/ml at 9 °C, 12 °C and 15 °C-fermentation temperature; comparison of pitching with 15, 30 and 45 Mio cells/ml at 9 °C.

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Tables and Figures see Appendix

C) Various numbers of yeast generations: 7 tests; 3 tests to compare beer resulting from 1st, 5th and 8th fermentation (fermentation temperature: 11 °C), 4 tests to compare 3rd and 11th fermentation (fermentation temperature: 12 °C). To get the requested number of generations in an acceptable period, wort was fermented at room temperature until reaching the respective number of generation (re-pitching after centrifugation of the yeast at 4 °C, 4000 r.p.m., 5 min.).

D) Pressure fermentation: 4 tests were conducted to compare normal fermentation (without pressure) with pressure fermentation. Pitching at 10 °C; pressure fermentation: Closing of the fermentation tanks after 24 h and further fermentation at 0,5 bar; 48 h after pitching: 18 ° C fermentation temperature in both approaches.

2.1 Polyphenol analysis

The haze relevant flavan-3-ols (Prodelphinidine B3, Procyanidine B3, Catechine and Epicatechine) and the phenolic acids (Vanillic-, Caffeic-, p-Coumaric-, Ferulic- and Syringic acid) were detected by HPLC. Sample preparation was done using solid phase extraction on polyamide (PA 1000 mg Macherey-Nagel). After conditioning the cartridge with 10 ml H_2O (10 min) the sample is added (40 ml). The next steps are to wash the polyamide-cartridge with 10 ml H_2O and to dry with N_2. Subsequently the polyphenols can be eluted with 2.5 ml of N,N-dimethylformamide solution (85 % v/v), (Sigma-Aldrich Taufkirchen). Last the eluate is filtered through a 0.45 µm filter and injected into the HPLC system.

The HPLC-System consists in a Perkin Elmer automatic sampler, 200 series, with a diode array detector (UV-detector). The column is a Lichrospher 100-5 RP-18 ec, 250 mm length (Macherey-Nagel).

HPLC conditions: Injection volume 20 µl, flow rate 0.8 ml/h, wavelength 275 nm, temperature 30 °C, solvent A: 1 % acetic acid (Merck Darmstadt), solvent B: 1 % acetic acid in acetonitrile (Merck Darmstadt). Gradient: 5 min. to 97.5 % A, 30 min. to 92.5 % A, 65 min. to 22 % A, 5 min. to 5 % A. All o the used reagents are HPLC-grade. The method is based on *Papp/Kusche* 2005 [11].

2.2 Proteins

Total soluble nitrogen according to MEBAK II 2.8.1.1.

Proteins, precipitable with Tannin (Pawlowski-Schild, Die Brautechnischen Untersuchungsmethoden, 8. Auflage, Verlag Hans Carl, Nürnberg 1961, pp. 175-177).

TCA-precipitable proteins were detected by a modified Biuret-test (own method, based on *Kadenbach* 1966) [12]. Precipitation of proteins in 10 ml of degassed (and centrifuged) beer with 2 g TCA (>99 %), (Roth Karlsruhe) at 0 °C for 1 h (permanent stirring); at least 30 min centrifugation at 0 °C (13000 r.p.m.); washing of the protein pellet with 5 ml cold acetone; 15 min centrifugation at 0 °C (13000 r.p.m.); Redisolving of the protein residue in 1 ml aqua dest using an ultrasonic bath (Bandelin Sonorex RK 100) for 30 min; incubation of 100 µl suspension with 900 µl Biuret-reagent (100 ml 0.2 M NaOH + 2.25 g $C_4H_4KnaO_6$ * 4 H_2O + 0.75 g $CuSO_4$ * 5 H_2O + 1.25 g KI filled up with 250 ml aqua bidest) for 10 min; photometric measurement at 546 nm (Photometer Ultrospec 3100 pro) against blank sample (100 µl aqua dest + 900 µl Biuret-reagent); calculation of the protein concentration with calibration curve, standard: Bovine serum albumin (BSA) (Roche Diagnostics, Mannheim).

2.3 Polysaccharides

The detection of high molecular polysaccharides based on the method according to *Plügler* 1905 [13] but was modified with regard to the application in beer. 5 ml beer and 3 ml hot KOH-solution (30 %, Merck Darmstadt) are put in a glass for centrifugation; heating in boiling water for 20 min. (open glass), then cooling with tap water; addition of 0.5 ml Na_2SO_4 (Merck Darmstadt) saturated solution and 10 ml ethanol (95 %, Sigma-Aldrich Taufkirchen); intensive mixing, then heating in hot water to the boiling of the alcoholic solution, cooling with tap water; centrifugation (15 min., 13000 r.p.m., room temperature), thorough decantation of the supernatant; washing of the residue with 5 ml ethanol (60 %, Sigma-Aldrich Taufkirchen) and centrifugation (15 min., 13000 r.p.m., room temperature); decantation of the supernatant; drying of the residue in a rotary evaporator at 42 °C; redisolving with 10 ml aqua bidest., then sample diluting (4:1 aqua bidest – sample solution); test tubes: A) blank sample (1 ml aqua bidest), B) standard 1 ml Glucose-solution (0.02 mg/ml, Sigma-Aldrich Taufkirchen), C) 1 ml diluted sample; cooling of the test tubes and addition of 2 ml Anthron-solution (200 mg Anthron/100 ml H_2SO_4(!)), then vortexing (Vortex-Genie Winn/Netherlands); heating for 15 min. at 90 °C, then cooling to room temperature; measurement of sample and standard in the photometer (Photometer Ultrospec 3100 pro) at 623 nm against the blank sample; concentration of high molecular polysaccharides:

$$C\ [mg/ml] = E_{Sample}/E_{Standard} * 0.02 * 0.9 * 10$$

2.4 Redoxpotential

Redoxpotential was measured by cyclovoltametric measurement as well as by an electrochemical variation of the Indicator-Time-Test using an electrochemical analyser (EAA). These methods have been precisely described at the 29th EBC Congress in Dublin [14].

2.5 Fermentation by-products

Higher alcohols and esters (MEBAK III 1.1.1/III 1,2,1)

SO_2 (MEBAK II 2.25.2)

2.6 Further beer analyses

Foam with LG-Foamtester (MEBAK II 2.19.3)

Scaba analysis for attenuation degree, residual extract, original gravity, pH

Total dissolved oxygen in the bottled beer was measured as standard control in the form of spot checks with a Digox 6 (Dr. Thiedig) (MEBAK II 2.37.3). Concentrations ranged from 0.1–0.3 mg/l. Within the conducted test-batches no deviations have been detectable.

Additionally a force test (40 °C/ 0 °C, MEBAK II 2.15.2.1) was performed over a period of at least 4 warm days in order to get information on the colloidal stability.

3 Results and discussion

A) Different fermentation temperatures

Two approaches, each repeated three times, were conducted to get information on the influence of different fermentation temperatures. The 2nd approach was done to get more detailed results concerning the haze relevant substances using a wider temperature spectrum.

Table 1 shows, as expected, faster fermentation performance at higher temperatures. It is possible to decrease the duration of fermentation from 7–8 days (9 °C) to 5 days (11 °C and 12 °C), respectively to 4 days (13 °C and 15 °C). No significant differences concerning the Scaba-Beer analysis can be found within the 2 approaches, except from the slightly higher pH in the cold fermented beer, induced by the lower pH-decrease during the first 48 hours of fermentation. Esters and higher alcohols increase constantly at fermenting with higher temperatures as opposed to the vicinal diketones that are reduced in a faster way. SO_2 seems not to be influenced by the variation of the temperature.

Figure 1 shows haze formation during the force test in the filtered (unstabilised) and bottled beer. All of the conducted approaches (only two figured) exhibited the same performance, namely a reproducible lower increase in haze in the beer resulting from cold fermentation. Fermentation at 13 °C and 15 °C results in worse colloidal stability. Differences according to the haze formation between the two approaches can be attributed to the different wort composition. Concentrations of haze relevant polyphenols and proteins are the crucial factor concerning the performance in the force test.

Of further interest is the colloidal haze (90°-measurement) in the fresh beer (warm day 0), depicted in figure 2. Obviously warm fermentation (>13 °C) is resulting in remarkably higher amounts of haze already in the beer coming out of the filter – this must be regarded as a decisive disadvantage of high fermentation temperatures, maybe due to polysaccharides (glycogen) released by the stressed yeast. In the range of 9 °C to 12 °C no significant differences were found.

One basic question was if there is a correlation between haze relevant polyphenols and proteins in the bottled beer and the different performance of haze formation, shown in Figure 1. Considering the flavan-3-ols (Figure 3) in the filtered beer, pictured as a sum of the flavan-3-ol- monomers and -dimers, it becomes apparent that there are no significant differences between the three samples, neither in approach 1 nor in approach 2. Even if the concentrations of flavan-3-ols are decreasing during fermentation, storage and filtration no essential influence by variation of fermentation temperature can be observed. Thus other substances or influencing factors seem to be responsible for the divergent haze formation. Nevertheless, considering all conducted tests, an obvious correlation between the basic flavan-3-ol concentration in wort and the intensity of splitting up-performance during the force test could be identified. The higher the levels of flavan-3-ols were the bigger was the influence of fermentation temperature on the colloidal stability.

In the case of the phenolic acids (Figure 4), also depicted as a sum, a slight trend becomes apparent with higher concentrations in the beer resulting from cold fermentation. Phenolic acids do not tend to influence haze formation in a direct way but may have a share in the antioxidative capacity and thus may act as indirect haze inhibitors by working against oxidative polymerisation. Usually phenolic acids are increasing during fermentation (not visible in approach 1), caused by the formation of vanillic- and caffeic acid by the yeast. Higher temperatures obviously induce a more intense degradation of the malt-deriving p-coumaric-, ferulic- and syringic acid but not of the yeast-produced phenolic acids which seem to be temperature-independent (not figured).

As opposed to the flavan-3-ols the measurement of haze relevant proteins (Figure 5) in approach 2 brought out a correlation to the force test in the warm fermented beers with rising concentrations of proteins which can be precipitated with Tannin as well as with the proteins detected by a modified Biuret method. Application of the Bradford-test according to total protein in approach 1 (not shown) emerged as too unspecific to detect haze-sensitive nitrogen. Therefore cold fermentation seems to cause lower contents of haze-sensitive proteins in the resulting beer, likely due to more intense precipitation and sedimentation during the longer fermentation period at lower temperatures.

One further investigation scope was the monitoring of the reducing power in the filtered beer. Figure 6 shows the total antioxidative capacity, received by the application of cyclovoltametric measurement (redoxpotential in mV). In this regard a higher potential means lower reducing power. Approach 1 shows a significantly better reducing power in the 9 °C-beer, which becomes worse at higher temperatures. The same tendency can be observed in approach 2, even if the differences are not significant. Thus, the better protection against oxidative polymerisation may also contribute to the lower haze in the cold fermented beers. In this regard, the possible correlation to the concentrations of antioxidative acting phenolic acids has to be pointed out. Contrarily the fast reducing substances, detected by an electrochemical indicator-time-test (Figure 7), exhibit other behaviour. The ITT-result (ascorbic acid equivalents) which is defined as the reducing power within a 4 minute-reaction time shows better (higher) reducing capacity in the warm fermented beers. Obviously higher fermentation temperatures result in higher concentrations of fast reducing substances – but this performance does not influence the total reducing power of the beer, consisting of fast and slow reacting antioxidants.

B) Different pitching rates

3 approaches were conducted to compare the influence of different pitching rates (15 Mio cells/ml and 30 Mio cells/ml, measured by using the Thoma cell-counter) by fermenting at 9 °C, 12 °C and 15 °C (approach 1-3). One further approach (approach 4) was done fermenting wort at 9 °C using pitching rates of 15, 30 and 45 Mio cells/ml. According to table 2 pitching with 30 Mio cells/ml accelerated the period of fermentation from 8 to 6 days (9 °C), from 5 to 4 days (12 °C) and from 4 to 3 days (15 °C). Pitching with 45 Mio cells/ml at 9 °C resulted in a time saving of 3 days in comparison to 15 Mio cells/ml. The Scaba-Beer analysis shows no differences concerning the measured parameters comparing the different pitching rates within the 4 approaches, except for approach 4, in which a higher pH can be observed in the 45 Mio cells-beer. Each approach was done with different wort which explains the general differences concerning attenuation degree and pH between approach 1–4. Higher pitching rates do not in any case lead to higher concentrations of esters and higher alcohols – in fact the approaches 2 and 4 show no differences. This performance is likely due to the different wort compositions and (final) attenuation degrees (very high in approach 2 and 4) as well as to the different physiological state of the pitching yeast. Vicinal diketones show no significant differences between the 15 and 30 Mio cells/ml – samples (approach 1–3), except from the lower 2,3-Pentandion concentration in approach 1 (30 Mio cells/ml). In contrast approach 4 exhibits increasing concentrations at higher pitching rates. Furthermore higher pitching rates seem to induce lower SO_2-contents in the beer (except for approach 1).

Figure 8 shows haze formation during the force test in the filtered (unstabilised) and bottled beer. Approach 1 and 2 exhibit slightly lower haze development in the beer fermented with 15 Mio cells/ml as opposed to approach 3 and 4, which shows the inverse performance. According to the experiences from approach A (different fermentation temperatures) this might be related to the higher concentrations of haze relevant polyphenols

(Figure 10) and proteins (Figure 12) which provide a wider influence by variations in fermentation control. At low concentrations of these substances obviously other influencing factors become more important, e.g. the redoxpotential (Figures 13 and 14). In this regard the unusual progress of haze formation in approach 4 is of particular interest - even if pitching with higher rates induces remarkably and inadmissibly higher amounts of haze in the fresh beer (Figure 9) coming out of the filter, haze development completely inverts after 2 warm days resulting in less haze than in the beer pitched with lower rates. This behaviour can be attributed to the significant higher reducing power of the beers resulting from higher pitching rates, which acts against oxidative polymerisation of polyphenols and proteins. As opposed to approach A (different fermentation temperature) both electrochemical tests show the same result, namely higher reducing power measured as total antioxidative power (Figure 13) as well as fast reducing power (Figure 14).

With respect to the flavan-3-ols (Figure 10) and to the phenolic acids (Figure 11) but also to the proteins (Figure 12) higher concentrations can be observed in the beers resulting from pitching with increasing rates. This effect might be attributed to these substances brought in by the yeast, but also to the decreasing time of fermentation resulting in lower precipitation and sedimentation of haze relevant substances working with higher pitching rates. Thus the better colloidal stability of the 15 Mio-beers compared to the 30 Mio-beers in approach 1 and 2 can be regarded as a result of the lower amount of the haze sensitive flavan-3-ols and proteins in these samples. Because of the generally high level of haze relevant substances in approach 1 and 2, the better redoxpotential of the 30 Mio-beers can obviously not impact colloidal stability in a decisive way.

The influence of fermenting with different pitching rates on colloidal stability depends on the redoxpotential but first of all on the concentrations of haze relevant substances. Nevertheless one further attribute of beer quality has to be considered – Figure 15 shows the correlation between foam stability and different pitching rates. A significant deterioration of foam becomes apparent working with higher amounts of yeast, likely due to an increasing activity of Proteinase A, which is known to act foam-destabilising [15,16].

C) Various numbers of yeast cycles

Two approaches, each repeated three and four times respectively, were conducted to highlight the effect of fermentation with various numbers of yeast generations. Aim of approach 1 was the comparison of the 1st, 5th and 8th cycle, whereas approach 2 concentrated on the 3rd and 11th cycle.

The repitched yeast did not decelerate the speed of fermentation in a remarkable way, at least not until the 11th cultivation. Scaba-Beer analysis as well as the detection of fermentation by-products (not measured in approach 2) did not bring out significant differences or tendencies within the 2 approaches.

The force test in both approaches (Figure 16) shows reproducible higher haze formation in the beers resulting from fermentation with severalfold repitched yeast than from yeast in early cultivations. This performance can not be attributed to haze relevant polyphenols (not figured), because no significant differences between the compared samples could be detected. Also the proteins and the total reducing power show just minimal but no significant tendencies in favour of the beer from earlier cycles (not depicted). As consequence these parameters can not serve as the main reasons for the distinct increase of haze formation. In contrast the detection of high molecular polysaccharides, conducted in the rows of approach 2, provided one possible explanation for this performance. Figure 17 points out lower amounts of unfermented polysaccharides in the 3rd-cycle beer compared to the 11th-cycle beer, which can also act as haze-relevant substances together with proteins and polyphenols. Perhaps the increasing amount of high molecular polysaccharides can be attributed to some natural yeast-modification (or mutation) during increasing numbers of yeast generations, resulting in a modified utilization of the wort-deriving sugars or to some high molecular reservoir- and/or reserve-substances accumulated by the stressed yeast. It has been reported that lower yeast growth rates (as observable at increasing numbers of generations) result in an accumulation of glycogen in the yeast cells [17,18]. Furthermore it has to be mentioned that no observable impact could be found concerning the haze measured in the fresh beer or according to the foam stability by fermenting with various numbers of yeast cycles, at least in the experiments conducted in this project.

D) Pressure fermentation

The application of pressure during fermentation allows the combination of fast fermentation at higher temperatures and low concentrations of fermentation by-products, induced by the inhibition of yeast growth. In this regard the crucial points are the regulation of the rising pressure concerning to the point of time (not to early) and to its extent of increase (not to high) [19]. The procedural method as done in this experiment is pictured in detail in chapter 3 (methods and materials). The basic question was if there is a detectable impact on the colloidal stability using this alternative of quick-performance fermentation.

4 approaches were conducted to compare the reference fermentation (without pressure) with pressure fermentation. Except for approach 4 the process of pressure-fermentation was not significantly more time-consuming than in the reference sample. Also the Scaba-beer analysis shows no remarkable differences within the 4 approaches (table 4), apart from the pH which exerts reproducible and significantly higher values in the pressure-beers, likely dependent on the less intensive decrease during main fermentation and to the release of basic amino acids and phosphates by the yeast [19]. Furthermore slightly higher alcohol-concentrations can be detected in the beers resulting from pressure fermentation. In this case only the SO_2-concentration was measured, as representative substance of fermentation by-products. As shown in table 4 the SO_2-content was remarkably lower after pressure-fermentation.

The force-test (Figure 18) in the filtered beers shows a slightly lower haze formation in the beers resulting from pressure-fermentation, except for approach 3 which exhibits the inverse performance. In general, pressure fermentation tends to result in an improved stability of the beers. This tendency can not be explained on the basis of the detection of the main haze forming substances – the flavan-3-ols (Figure 19) as well as the Tannin-precipitable proteins (Figure 20) show no differences between the reference and the pressure-sample in the filtered beer. In fact pressure fermentation obviously brings out higher contents of total soluble nitrogen, due to the inhibited utilisation of free amino nitrogen (FAN, Figure 20). Also the higher concentrations of high molecular polysaccharides (Figure 20) in the pressure beer might be due to inhibited utilisation-mechanisms or rather to high molecular reservoir- and/or reserve-substances accumulated by the stressed yeast. Nevertheless these conditions do not seem to influence neither haze formation nor foam stability (not pictured) in a decisive way. In spite of lower concentrations of the antioxidant acting SO_2 in the pressure beers also the total redoxpotential and the fast reducing power (not depicted) show no significant differences compared to the reference sample. The effect of lower SO_2-concentrations might be compensated by higher

concentrations of other reducing substances in the pressure beer, e.g. the phenolic acids (Figure 19). Thus, there must be some other reason for the pressure beers tending to a better colloidal stability – most likely this performance can be attributed to the remarkably higher pH-level in combination with slightly higher concentrations of alcohol which may act haze-inhibiting. According to Siebert the maximum haze can be observed at a pH of 4,2 – above this level haze decreases. Minimum haze is observed at 5,5 vol% alcohol, above and below this level haze increases [5,6,20,21].

4 Conclusions

The aim of this project was to evaluate four different variations of fermentation-controls with the main focus on colloidal stability and reducing power of the resulting beers. It became apparent that it is possible to influence these parameters by fermenting at different temperatures, with different pitching rates, by using yeast from various numbers of generations as well as by the application of pressure fermentation. In the following the effects and the suspected reasons for the respective performance will be elucidated at a glance.

Fermentation at higher temperatures enables a faster fermentation progress but induces higher concentrations of fermentation by-products and causes inferior colloidal stability of the resulting beers and higher levels of colloidal haze already in the fresh beer. The main reasons for the faster haze formation in the warm fermented beers are higher concentrations of haze-relevant proteins and the minor antioxidative capacity (total reducing power). Concerning colloidal stability, cold fermentation (9 °C) has emerged as the most appropriate method.

Pitching at increasing rates results in an accelerated fermentation period but also in inferior foam stability and higher amounts of colloidal haze in the fresh beer, most likely induced by higher concentrations of haze relevant proteins, polyphenols and polysaccharides. In contrast, further haze formation does not exhibit significant differences compared to a beer resulting from normal pitching (15 Mio cells/ml). This performance might be due to the remarkably superior reducing power in the intensively pitched beers which improves stability.

Fermentation with yeast from increasing generation-numbers does not decelerate fermentation period and has no obvious influence on fermentation by-products, on foam stability or on the reducing power, compared to beers resulting from earlier generations. Nevertheless colloidal stability becomes worse fermenting with yeast from increasing numbers of generations, likely due to higher concentrations of high molecular polysaccharides accumulated by the repitched yeast.

Pressure fermentation, as an alternative of quick-time fermentation, has no obvious impact on the duration of fermentation, on the reducing power or on foam stability but seems to improve colloidal stability of the resulting beers in a slight manner, compared to the reference beer (fermentation without pressure). This performance can not be attributed to the haze relevant polyphenols and proteins (no differences between reference- and pressure beer) but rather to the higher pH and the slightly higher alcohol concentration in the pressure beer which act as inhibitors against haze formation. Considering also the decreasing amount of fermentation by-products, the usage of pressure can be regarded as an interesting and promising approach in the range of beer fermentation.

It has been shown that fermentation control has an evident influence on the quality of the resulting beers, the reducing power and the colloidal stability to mention a few. The affect of fermentation is generally due to the concentrations of haze-relevant polyphenols and proteins brought in by the raw materials. The higher the amount of these substances the wider the influence of fermentation control. Resistance against haze formation can be improved by fermenting at lower temperatures (not higher than 12 °C) and by using yeast, which was not repitched more than about 3 times at best. For breweries preferring quick fermentation the application of pressure can be regarded as an adequate compromise. However it is not possible to get a colloidal stable beer over a long period only by optimising the process of fermentation. There are some other crucial factors, first of all the well directed selection of raw materials but also the processes in the brewing house. Nevertheless optimising the fermentation performance and pitching technology can be regarded as one further promising step to improve colloidal stability in a technological way.

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Acknowledgement

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Appendix

Table 1: Fermentation period, Scaba-Beer analysis and fermentation by-Products

	1st approach			2nd approach		
	9°C	**11°C**	**13°C**	**9°C**	**12°C**	**15°C**
Fermentation period [d]	7	5	4	8	5	4
Scaba-Beer analysis						
Alcohol [g/100g]	4,2	4,1	4,1	4,1	4,1	4,1
Residual extract [g/100g]	1,8	1,8	1,6	2,3	2,2	2,1
Attenuation degree [%]	85	85	86	82	83	83
pH	4,27	4,18	4,18	4,25	4,22	4,18
Fermentation by-Products in beer [mg/l]						
Esters	20,8	23,5	27,2	15,3	18,7	25,3
Higher alcohols	66,1	77,8	85,6	66,1	78,2	89,4
Total Diacetyl	0,05	0,03	0,03	0,04	0,03	0,03
Total 2,3 Pentandione	0,04	0,02	0,02	0,05	0,04	0,01
SO2	7	6	7	5	4	4

Table 2: Fermentation period, Scaba-Beer analysis and fermentation by-Products

	Approach 1 - 9°C		Approach 2 - 12°C		Approach 3 - 15°C		Approach 4 - 9°C		
Mio cells /ml	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**	**45**
Fermentation period [d]	8	6	5	4	4	3	8	6	5
Scaba-Beer analysis									
Alcohol [g/100g]	4,1	4,2	4,1	4,1	4,1	4,2	4,0	4,1	4,3
Residual extract [g/100g]	2,3	2,2	1,34	1,49	1,96	1,84	1,73	1,72	1,73
Attenuation degree [%]	82	83	89	87	84	84	85	86	86
pH	4,25	4,26	4,29	4,27	4,46	4,48	4,36	4,38	4,46
Fermentation by-Products in beer [mg/l]									
Esters	15,3	22,3	26,5	24,3	22,8	30,7	16,5	16,1	15,6
Higher alcohols	66,1	72,2	83,1	83,5	74	86,3	63,2	63,8	63,6
Total Diacetyl	0,04	0,05	0,05	0,04	0,04	0,04	0,02	0,03	0,04
Total 2,3 Pentandione	0,05	0,01	0,04	0,03	0,02	0,02	0,01	0,02	0,03
SO2	5	6	7	5	8	5	6	5	4

Table 3: Fermentation period, Scaba-Beer analysis and fermentation by-Products

	Approach 1 - 11°C			Approach 2 - 12°C	
Yeast cycle	**1st**	**5th**	**8th**	**3rd**	**11th**
Fermentation period [d]	7	8	8	5	5
Scaba-Beer analysis					
Alcohol [g/100g]	4,1	3,9	3,9	3,8	3,9
Residual extract [g/100g]	1,9	2,2	2,1	2,3	2,3
Attenuation degree [%]	84	81	82	80	80
pH	4,22	4,24	4,22	4,30	4,24
Fermentation by-Products in beer [mg/l]					
Esters	24,3	22,0	22,8		
Higher alcohols	77,2	73,8	77,7		
Total Diacetyl	0,03	0,02	0,02		
Total 2,3 Pentandione	0,02	0,02	0,02		
SO2	6	13	10	4	5

Table 4: Fermentation period, Scaba-Beer analysis and fermentation by-Products

	1st Approach		2nd Approach		3rd Approach		4th Approach	
	Ref.	**Pressure**	**Ref.**	**Pressure**	**Ref.**	**Pressure**	**Ref.**	**Pressure**
Fermentation period [d]	6	7	5	5	5	5	4	7
Scaba-Beer analysis								
Alcohol [g/100g]	3,9	4,1	4,1	4,2	3,8	3,9	3,9	4,1
Residual extract [g/100g]	2,4	2,0	2,0	2,1	2,0	1,9	2,2	2,1
Attenuation degree [%]	80	83	83	83	82	83	81	82
pH	4,47	4,58	4,42	4,52	4,42	4,55	4,36	4,58
SO2	9	7	10	6	7	4	5	3

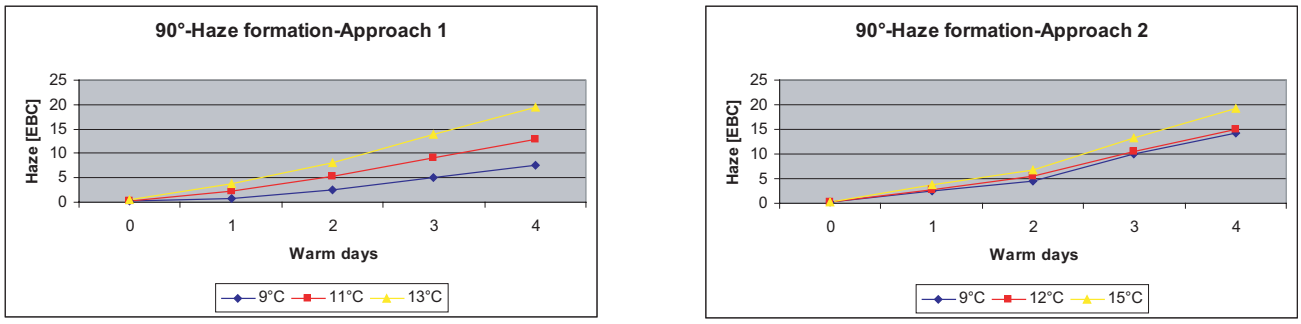
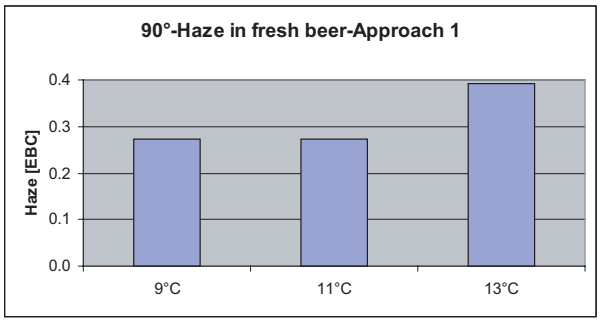


Fig. 1 90°-Haze formation in the force test



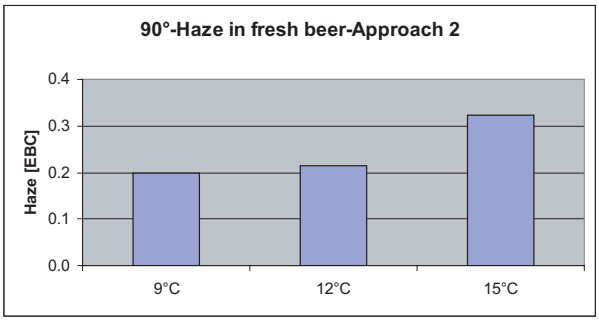
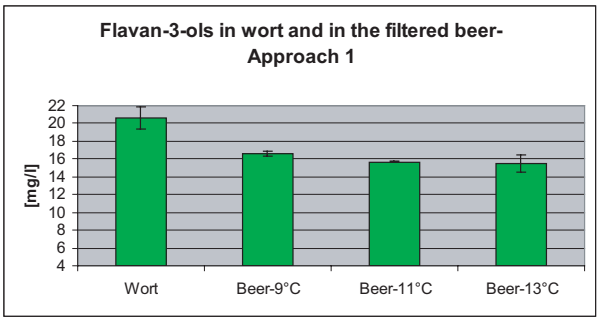


Fig. 2 90°-Haze formation in the fresh beer after filtration



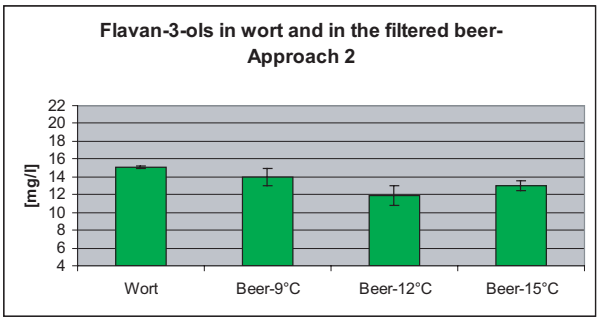
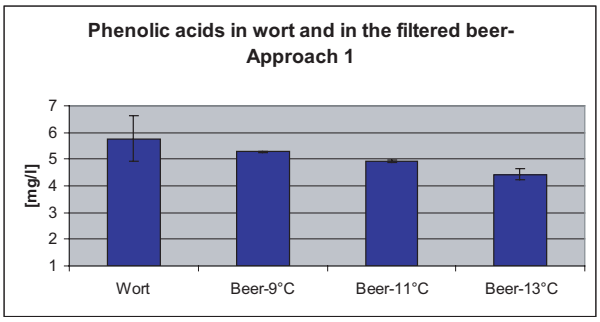


Fig. 3 Flavan-3-ols in wort and in the filtered beer



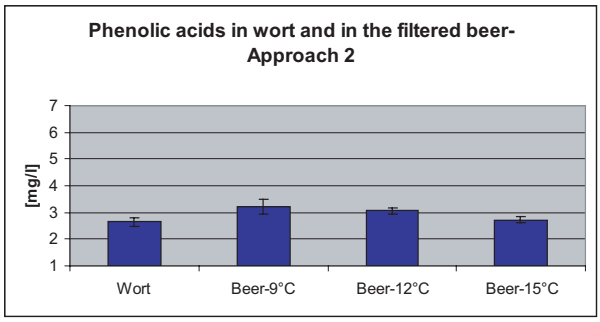


Fig. 4 Phenolic acids in wort and in the filtered beer

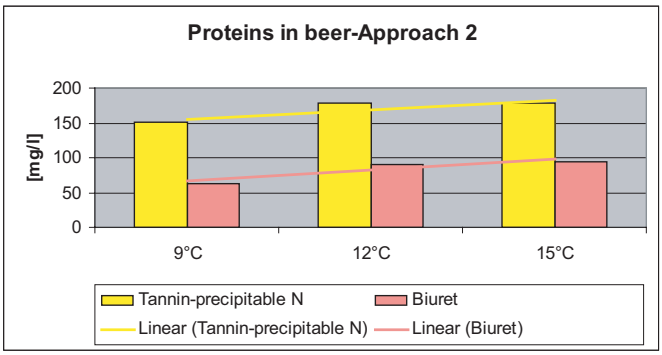
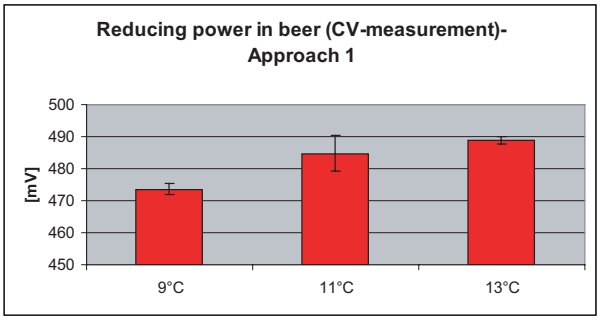


Fig. 5 Proteins in the filtered beer



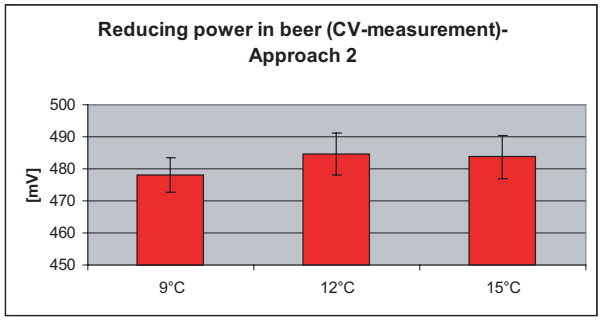
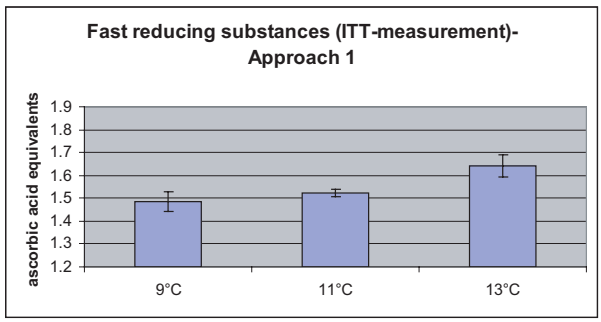


Fig. 6 Total reducing power in the filtered beer



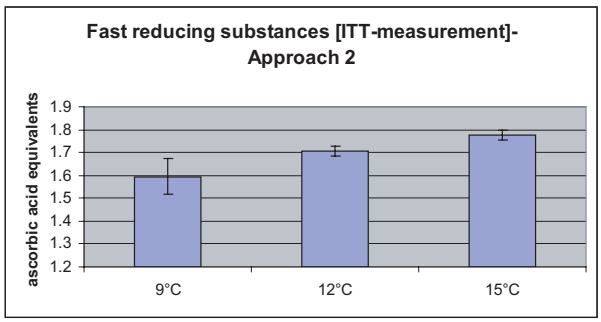
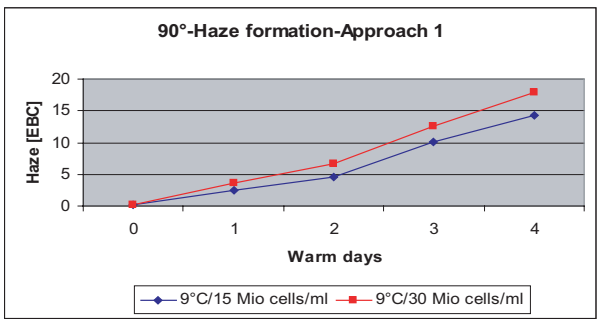
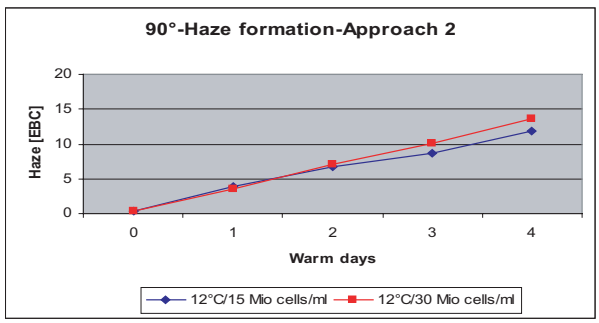
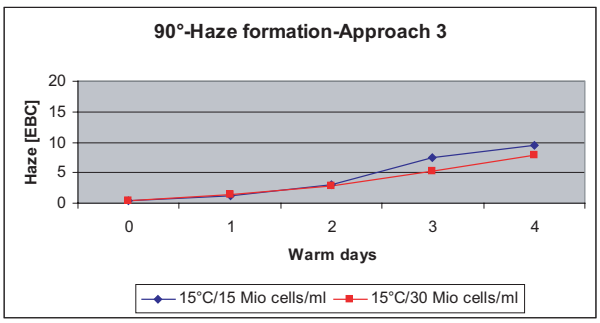


Fig. 7 Fast reducing substances in the filtered beer







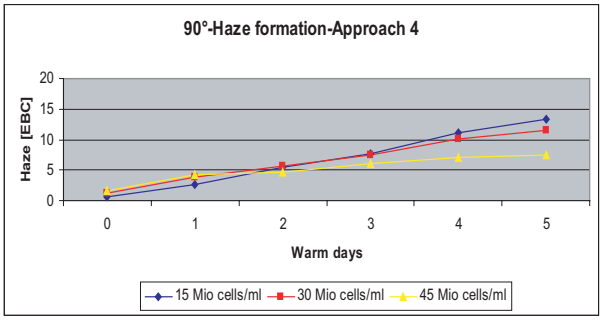


Fig. 8 90°-Haze formation in the force test

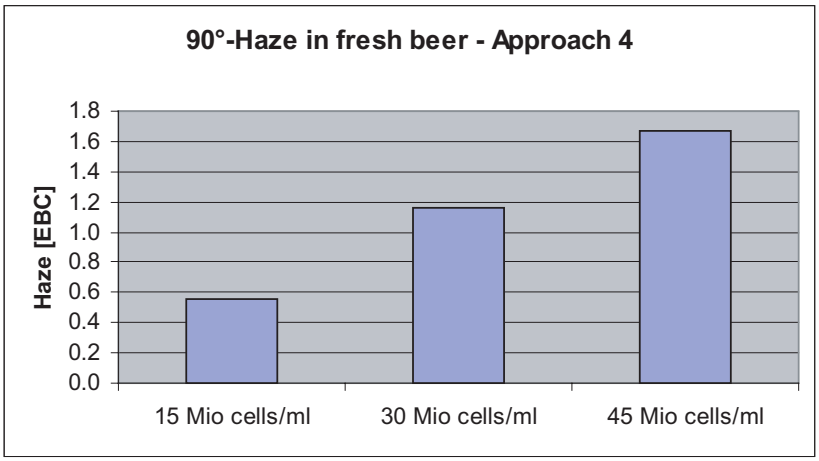
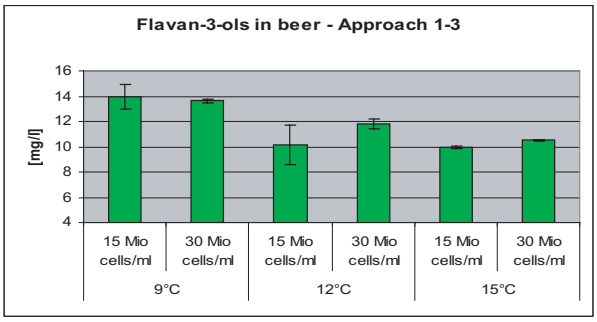


Fig. 9 90°-Haze in the fresh beer after filtration



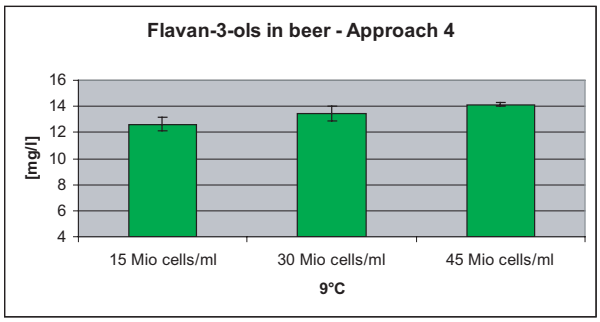
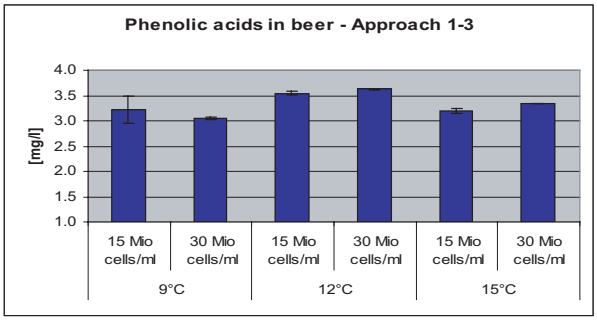


Fig. 10 Flavan-3-ols in the filtered beer



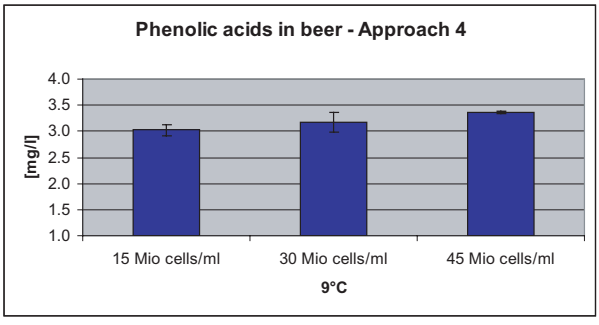
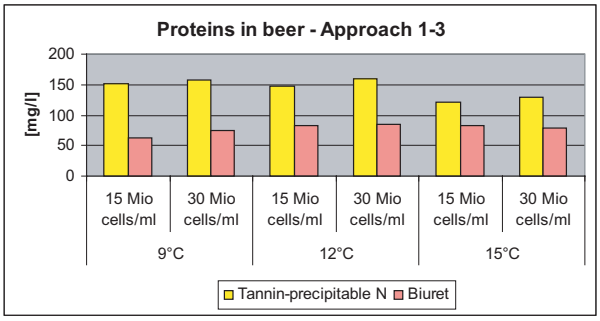


Fig. 11 Phenolic acids in the filtered beer



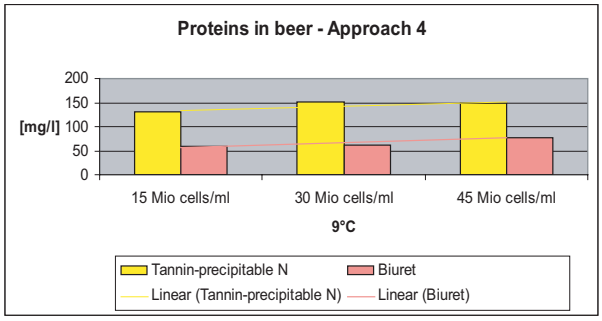
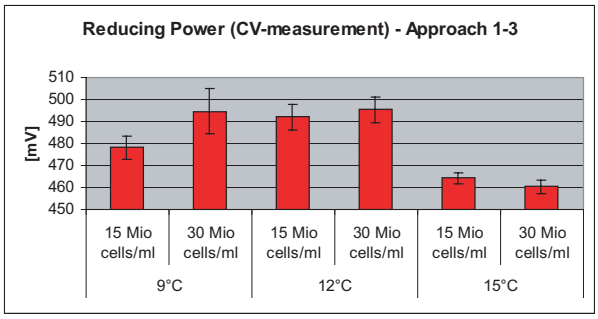


Fig. 12 Proteins in the filtered beer



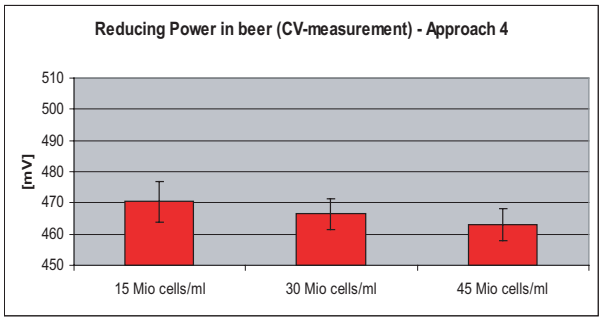
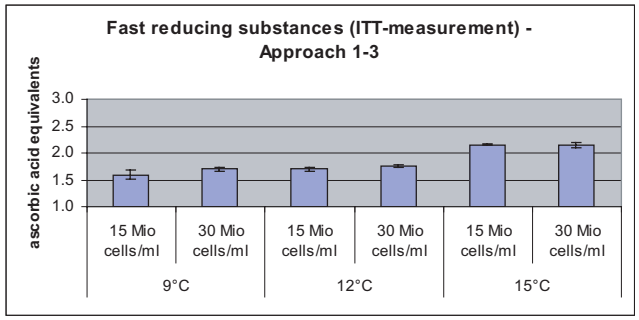


Fig. 13 Total reducing power in the filtered beer



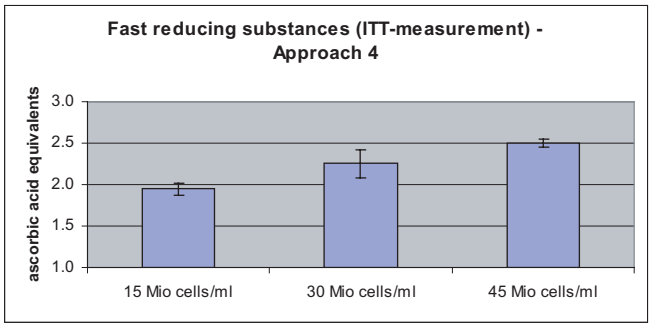


Fig. 14 Fast reducing substances in the filtered beer

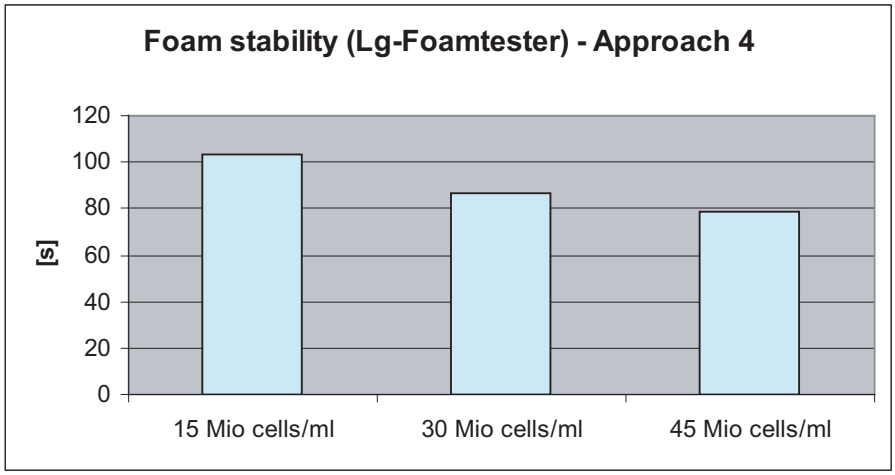
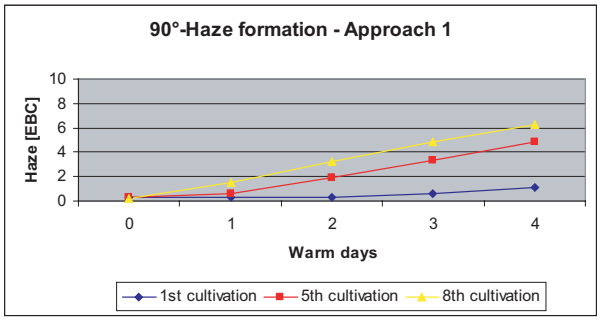


Fig. 15 Foam stability



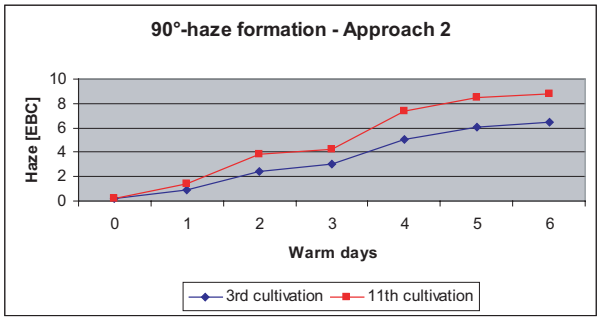


Fig. 16 90°-Haze formation in the force test

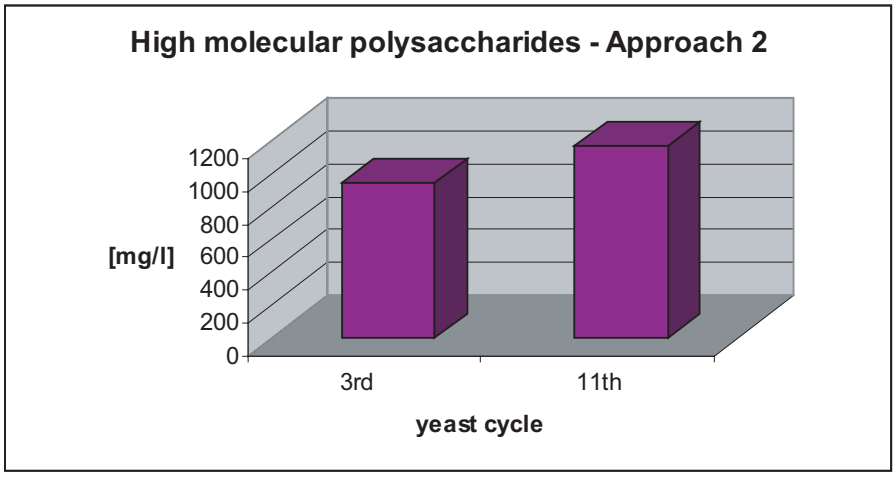


Fig. 17 High molecular polysaccharides in approach 2

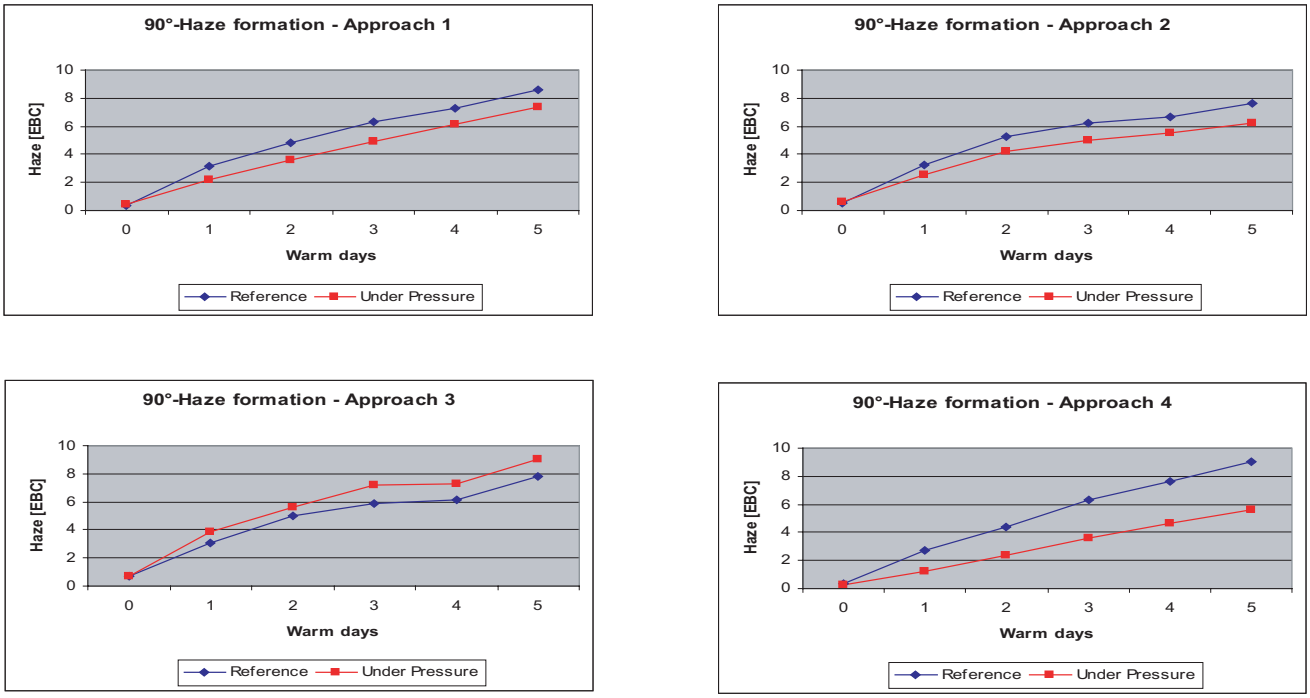


Fig. 18 90°-Haze formation in the force test

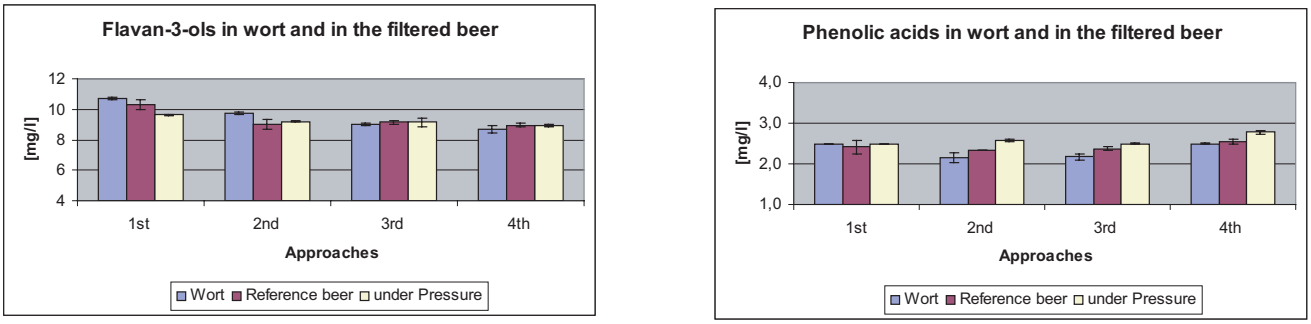


Fig. 19 Flavan-3-ols in wort and in the filtered beer

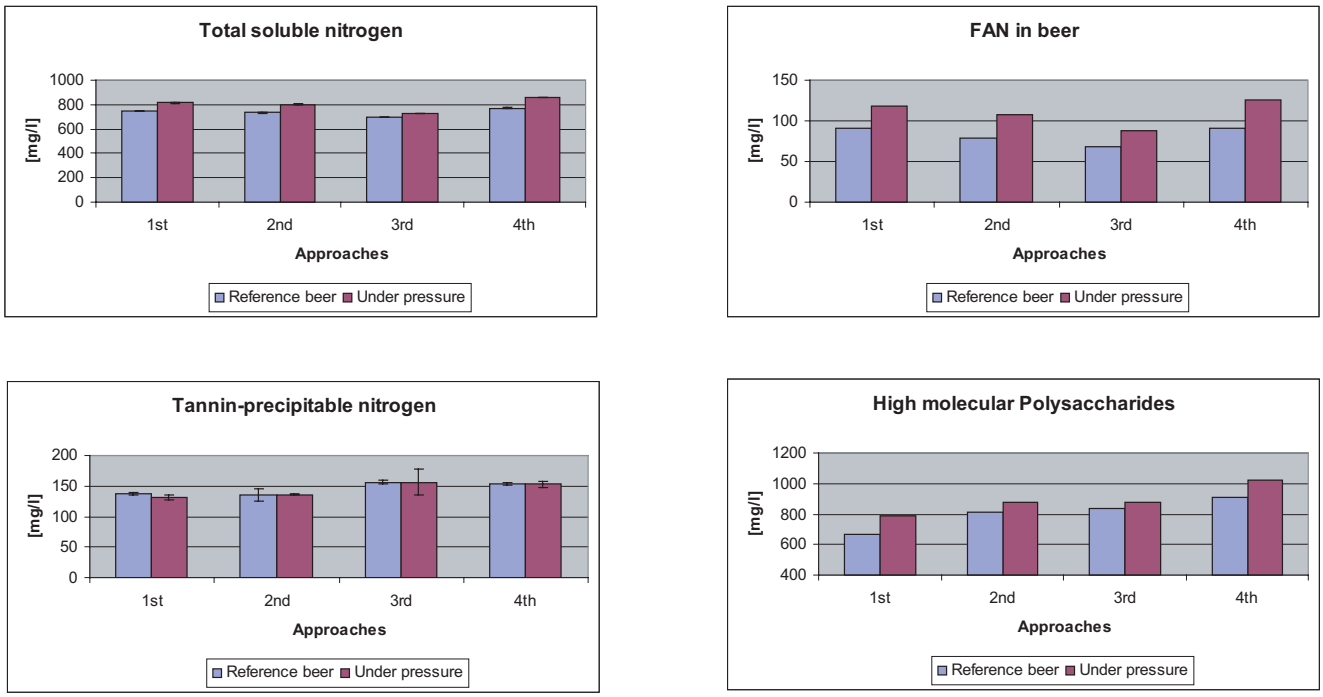


Fig. 20 Proteins and high molecular polysaccharides in the filtered beer