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Upstream Beer Stabilisation during Wort Boiling by Addition of Gallotannins and/or PVPP

Addition of stabilisation products in the upstream brewing process is a very convenient way of physico-chemical stabilisation without the need for extra filtration or the risk of beer losses. Therefore, in this study the use of appropriate stabilisation products upstream the brewing process, more specifically at the end of wort boiling, have been evaluated in relation to improved colloidal stability. Applications of PVPP (Polyclar 10, ISP) and gallotannins (Beerotan Q, BFTI) have been investigated. The lowest gallotannin levels (wort boiling: 5 g/hL; contact time in boiling kettle: 3 minutes) are already sufficient to obtain enhanced stability due to adequate removal of haze-sensitive proteins. Furthermore, the addition of 10 g/hL PVPP has an explicit effect on the amounts of polyphenols, which results in an improved colloidal stability. Lowering pH at mashing-in also results in improved physico-chemical properties and flavour stability.

Descriptors: colloidal stability, haze, wort boiling, gallotannins, PVPP

1 Introduction

Preserving colloidal stability in lager beers is a difficult issue for the brewing industry. Interactions between haze active polyphenols (proanthocyanidins) and proteins can result in irreversible bounding which has a negative impact on the 'shelf life' of beer. Also polysaccharides, metal ions and minerals can be responsible for the forming of haze. The composition of the raw materials is a first important parameter [16, 22]. During mashing, wort boiling, fermentation and maturation, haze can be formed and removed. The pH is a critical factor in obtaining maximum protein precipitation. To improve hot break removal after wort boiling, the pH of the wort should be between 5.0 and 5.2. *Lermusieau* [13] showed that clear worts were obtained in the whirlpool for pH 5.0 and 5.2. For pH higher than these values the clarity was not acceptable.

Gallotannins are known to act as radical scavengers, metal-chelating agents and anti-oxidants [2, 29]. Besides these characteristics they are also very effective in coagulation and flocculation of thiol-containing proteins [10]. According to *Aerts et al.* [2] the flocculation of proteins is supported by lowering the pH of the brewing water, which explains the positive effects of pH 5.2 at mashing-in on colloidal stability.

Colloidal instability in beer is caused mainly by interactions between polypeptides and polyphenols; this has already been reported by several authors [11, 12, 14, 16, 22, 25, 27]. The natural haze active polyphenols in beer are mainly proanthocyanidins because of their relatively large and complex structure. A haze active polyphenol binds at least with two proteins. The haze forming capacity of those proteins is dependent on their proline content [28].

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Tables and figures see Appendix

Next to the problem of colloidal instability the request for freshness of beer is a high standard in the brewing industry. Not only should a fresh beer have a pleasant taste, it should also keep its original flavour and freshness in spite of the treatment with stabilising agents. According to Aerts et al [3] a combination of pH 5.2 and a temperature of 63 °C at mashing-in results in a prolonged flavour stability and physical stability of the beer.

The use of stabilising agents, in addition to the adequate selection of raw materials, can improve the shelf life of beer. Addition of stabilising agents to remove haze active polyphenols or proteins can result in improved colloidal stability of beer.

Stabilisation costs and filter aids may be seriously diminished by using appropriate stabilisers in the upstream process. Addition of gallotannins at mashing-in and during sparging already has been reported to result in an improved flavour and physical stability of the beer [1,2]. This paper presents results demonstrating the effectiveness of the approach of using appropriate stabilisation products upstream in the brewing process, more specifically at the end of wort boiling and the influence of mash acidification in combination with the use of the stabilisation products.

2 Material and methods

2.1 Preparation of beers on 50 L scale

To examine the potential of gallotannins (Beerotan Q) and polyvinylpyrrolidone (PVPP; Polyclar 10) as adsorption agents for respectively haze-active proteins and polyphenols, six brewing trials (50 L scale; 12 °P worts, mashing-in 5.6) were performed in the first series (see Table 1) and four brewing trials (50 L scale; 12 °P worts, mashing-in 5.2) in the second series (see Table 2). The pH was measured and controlled with a pH electrode in situ. Lactic acid was added to adjust the pH of the mash.

All beers were brewed with 9.56 kg of dry coarse (two-roller) milled pilsner malt (Bavaria Malt, The Netherlands) and 36 litre of

reverse osmosis water with addition of 40 ppm Ca^{2+} for the reference beer and 60 ppm Ca^{2+} for the beers stabilised with gallotannins. It proved important to add extra calcium at mashing-in in order to compensate for the expected chelating effect of gallotannins [28]. The brew scheme is as followed: 63 °C (30 min), 72 °C (20 min), 78 °C (80 to 100 min, including wort filtration with lautertun); during 60 minutes of wort boiling, gallotannins (Beerotan Q, Belgian Fine Technology International, Belgium) and PVPP (Polyclar 10, ISP, England) were added at the end of the boiling process; next to the stabilising agent also 0.2 ppm Zn^{2+} and isomerised hop extract were added at the end of boiling; wort clarification achieved with whirlpool. The obtained worts (original gravity 12 °P) were fermented at 12 °C during 8 days, followed by maturation at -1 °C during 14 days. The beer was filtered using kieselguhr/cellulose sheets (1 µm) and bottled with automatic filling and crowning using a rotating 6 head counter pressure filler with double pre-evacuation (CIMEC) in brown glass bottles (25 cl) to obtain O_2 -levels under 50 ppb.

2.2 Beer analysis

Finished beers prepared as described above were compared by measuring various beer quality parameters. The following analysis were carried out according to EBC methods [19], IOB methods and other published procedures: total polyphenols: EBC method 9.11; flavanoid content: EBC method 9.12; proanthocyanidin content: Bate-Smith (1973); sensitive proteins: IOB-method 9.37; reducing power according to TRAP assay [5] and DPPH test [9]. Alcohol (ml/100 ml), original extract (g/100g), real extract (g/100g) and final attenuation (%) were measured using an Anton Paar Alcolyser with a DMA 5000 density meter (Anton Paar, Austria).

2.3 Haze measurement

Haze measurements were performed on beer samples using a Haffmans haze meter. Results are reported in EBC formazin units. The instrument was calibrated with suitable turbidity standards. Chill haze was measured in bottled beers after overnight storage at 0 °C. Permanent haze was measured 24 hours later after storage at 20 °C.

2.4 Evaluation of beer flavour stability

Sensory quality of the beers was investigated on both fresh samples and after forced-ageing. Samples of bottled beer were forced aged at 40 °C for 5, 10, and 15 days, respectively. The staling degree of the samples was evaluated by a panel of 8 tasters from our brewing department. A scale of 0–10 was used for determination of the degree of staling: 0 = fresh, staling not detectable; 2 = very slightly stale; 4 = slightly stale; 6 = strongly stale; 8 = very strongly stale, undrinkable [5].

2.5 Residue analysis by HPLC

The presence of residual gallic acid in the beers was evaluated by HPLC. Beer samples were degassed and tannase (100 mg/L) was added. Incubation of the sample was performed at 25 °C for 2 hours under stirring (300 rpm). Next, the pH was adjusted to pH 2 using HCl (2 mol/L) and the acidified preparation was extracted with ethyl acetate. Ethyl acetate was removed by rotary

evaporation and the residue was re-dissolved in 1 ml of methanol. Prior to HPLC analysis, the extract was filtered through a 13 mm HPLC syringe filter (0.2 µm PTFE). Gallic acid was quantified using reversed-phase HPLC (Merck Hitachi Liquid Chromatography) on an Alltima C18 5 µ column (250 x 4,6 mm). The mobile phase consisted of solvent A (formic acid and water; 1/99; v/v) and solvent B (acetonitrile and methanol; 5/95; v/v). Separations were performed by gradient elution (0 min: 100 % A; 24 min: 80 % A /

20 % B; 34 min: 100 % B; 48 min: 100 % A; 52 min: 100 % A) at a flow rate of 0.9 ml/min. Detection was carried out at 280 nm (UV-detector L-7400).

3 Results

3.1 Evaluation of beers brewed on 50 L scale with addition of PVPP or gallotannins during wort boiling

The results of the standard analyses on the reference beer A and on the beers with addition of stabilisation products during wort boiling, i.e. gallotannins for the beers B and C, and PVPP for the beers D and E, respectively, are summarised in table 3. Also the results for beer F prepared with the combined treatment of both gallotannins and PVPP are represented in table 3.

3.1.1 Addition of gallotannins as stabilising agent in the upstream brewing process

Polyphenols and phenolic acids, such as gallotannins, play an important role in the flavour stability because of their antioxidant activity. Gallotannins will precipitate with sensitive proteins to minimize their levels in beer. Proline sites in these haze-forming proteins bind to the gallotannins so that they are selectively adsorbed. Foam proteins contain little proline and are therefore not significantly affected by a treatment with gallotannins [12].

Application of gallotannins upstream in the brewing process, more specifically 3 minutes before the end of wort boiling, has a large impact on the amount of sensitive proteins in the final beer. Addition of 5 g/hL gallotannins resulted in a relative decrease in sensitive protein of about 54 %, whereas an addition of 10 g/hL gave a relative decrease of even 70 % compared to the reference beer A.

Associated with a higher level in total polyphenols in the treated beers, the beers B, C and F (beer F: addition of both gallotannins and PVPP) also show a significantly higher reducing power in comparison with the reference beer A (see Table 3, results obtained by TRAP and DPPH assays). The increase in the amount of polyphenols by a gallotannin treatment results in only a small amount of gallic acid residue (HPLC detection, Table 5).

The colour intensity of the gallotannin beers is lower than that of the reference beer.

The results of the standard analyses on the reference beer G and on the beers, with addition of gallotannins during wort boiling, H

and I are summarised in table 4. This table shows the impact of pH adjustment at mashing-in (pH 5.2). Also the results for beer J prepared with the combined treatment of both gallotannins and PVPP are represented in table 4.

Figures 1 and 2 show the impact of a gallotannin treatment on the level of polyphenols and the reducing activity measured by the TRAP method (Fig. 1) and the DPPH method (Fig. 2). The higher reducing activity of the beers treated with gallotannins can be linked to the higher concentrations of polyphenols. The brewing trials (especially brewing trials H and I) with pH adjustment at mashing-in show a slightly larger increase of TRAP value in correlation to the brewing trials with mashing-in at pH 5.6.

3.1.2 Addition of PVPP as stabilising agent in the upstream brewing process

PVPP is used as stabilising agent because of its ability to bind with haze active polyphenols. PVPP has a structure similar to peptidically linked proline chain [11, 12, 15, 26]. Addition of PVPP has an explicit effect on the amounts of polyphenols in the finished beers [12]. As shown in table 3 and figure 3, application of PVPP at the end of wort boiling is related to a considerable decrease in total polyphenols, flavanoids, and haze-active proanthocyanidins in the finished beers. A pronounced decrease in haze-active proanthocyanidins, i.e. relatively

40 % compared to the reference beer, can already be noticed after a short contact time of 3 min during wort boiling. A contact time of 5 min results in a less efficient removal of the haze-active proanthocyanidins. The effect of PVPP treatment is also expressed by the strong decrease in reducing power of the resulting beers D and E (see Table 3, results obtained by TRAP and DPPH assays). This may result in a diminished flavour stability of these beers. Flavanoids in beer are expected to scavenge active oxygen species and prevent the oxidation of beer components during storage [21]. According to *Mikyska et al.* [16], both malt and hop polyphenols suppress formation of ageing carbonyls during the brewing process and upon beer storage. In particular polyphenols such as catechin and procyanidin B-3 (both flavanoids) act as powerful antioxidants, thereby protecting other components towards oxidation [16, 30, 31]. Finally, levels of sensitive proteins also seem to be affected in the finished beers by the treatment with PVPP at the end of wort boiling.

3.1.3 Addition of both gallotannins and PVPP in the upstream brewing process

Figure 4 shows the relative evolution of the haze active components and the reducing capability in beers stabilised with both stabilising agents during the upstream brewing process. This evolution is reported in relation to the reference beer. Besides the impact of the stabilising actions also the impact of the pH at mashing in is presented.

The decrease in the amounts of sensitive proteins is more powerful in beers produced with pH adjustment at mashing-in, but those beers shows a higher level of proanthocyanidins in relation to stabilised beers without pH adjustment. All stabilised beers have

an improved reducing activity which can result in prolonged flavour stability.

3.2 Quantitative HPLC-profiling of gallic acid

Table 5 shows the amounts of gallic acid present in the fresh beers. As expected, the amount of gallic acid residue increases as a function of gallotannin dosage. However, application of gallotannins during the upstream process only results in a small amount of residue in the final beers.

3.3 Sensory evaluation of the beers

Results of the sensory evaluation of the beers are presented in table 6. All fresh beers were positively evaluated by the tasting panel, in that the panel did not recognize any degree of ageing. However, the beers with addition of gallotannins at the end of wort boiling were preferred because of their higher sensorial freshness.

After 5 days of storage at 40 °C, beers brewed with gallotannins were rated with a lower ageing score than other beers; both the reference beer A and the beers stabilised with PVPP (D and E). After 10 and 15 days at 40 °C, beers were evaluated negatively by the tasting panel, both the reference beer and the stabilised beers. Ageing scores varied from 4 (slightly staled) to 6 (strongly staled). Nevertheless, regardless of the aging period, the gallotannin beers always received the lowest overall ageing score, which could be related to their higher reducing power. Positive effects of gallotannins on beer flavour stability have been reported previously [1, 2, 22].

The addition of PVPP in the upstream process has no negative effect on the beer taste of fresh beer. Despite of the lower reducing capacity of the beers treated with PVPP, in correlation to their reference, the ageing scores were similar as the reference beer.

3.4 Shelf life in correlation to forced aging

The nature of protein-polyphenol complexes is reversible in the early stages of their formation. This expresses itself as chill haze which can be dissipated by warming the beer to room temperature [23, 28]. Permanent haze originates from protein-polyphenol interactions that are irreversible. The shelf life of the final beers is expressed as the chill haze determination in correlation to the amount of day's storage at 40 °C.

Measurements for the chill haze taken at 5 day intervals during warm storage at 40 °C are given in figures 5 and 6. The forced shelf life resulting from the treatment was calculated by using the time required for total haze to increase to 2 EBC units.

The critical value for stored beers is 2 EBC.

3.4.1 The evolution of chill haze development in the beers produced without pH correction during mashing-in (pH is 5.6)

As a criterion to evaluate the impact of the different treatments on the shelf life, the time needed for the haze to increase to 2 EBC units, was used. The following values were derived:

- Beer A (reference beer): 7.21 days;
- Beer B (5 g/hL gallotannins, 3 min contact time): 17.23 days;
- Beer C (10 g/hL gallotannins, 3 min contact time): 15.82 days;
- Beer D (10 g/hL PVPP, 3 min contact time): 13.90 days;
- Beer E (10 g/hL PVPP, 5 min contact time): 13.50 days;
- Beer F (10 g/hL gallotannins and 10 g/hL PVPP): 15.53 days.

These results demonstrate that a concentration of 5 g/hL gallotannins is more effective than 10 g/hL PVPP appears to be somewhat more effective when applying a smaller contact time. Clearly, treatment with low amounts of gallotannins at the end of wort boiling results in a beer with a better colloidal stability.

3.4.2 The evolution of chill haze development in beers produced with pH 5.2 at mashing in:

- Beer G (reference beer): 14.28 days;
- Beer H (5 g/hL gallotannins, 3 min contact time): 23.31 days;
- Beer I (10 g/hL gallotannins, 3 min contact time): 27.06 days;
- Beer J (10 g/hL gallotannins and 10 g/hL PVPP): 29.89 days.

The results in figure 6 illustrate the impact of the pH at mashing-in on the evolution of chill haze development. The brewing trials with pH adjustment at mashing-in (pH 5.2) show an explicit improvement of the shelf life in function of forced ageing at 40 °C. In combination with pH adjustment at mashing-in a higher dosage of gallotannins is more recommended because of the longer storage time.

Beer J has the longest shelf life when looking at the data on forced ageing at 40 °C, which indicates that it is very rewarding to combine mashing-in with pH adjustment and applications of both PVPP and gallotannins during the boiling process.

4 Conclusion

In general, the use of stabilisation products during wort boiling, more specifically at the end of the boiling process, has a positive impact on the shelf life of the final beers. The sufficient removal of the haze-active polyphenols by a PVPP treatment (10 g/hL and 3 minutes contact time) has a positive impact on the colloidal stability, and thus on the shelf life, of the finished beer. The sensory evaluation of the stabilised beers has shown that PVPP treatment does not deteriorate the flavour stability, which is in contrast with the results on the analytical evaluation of the reducing capacity.

pH adjustment (5.2) at mashing-in, combined with application of both PVPP and gallotannins in the upstream brewing process, seems promising because of the explicitly prolonged shelf life.

Addition of gallotannins in the boiling kettle at the end of the boiling process results in an improved physical stability. They also contribute to a better antioxidative capacity and a prolonged flavour stability. The beers brewed with addition of gallotannins at the end of wort boiling were preferred by the taste panel because of their higher sensorial freshness and fullness.

In view of the obtained results discussed above, it is possible to produce a beer with long shelf life and improved flavour stability by addition of gallotannins in the upstream brewing process. Application of gallotannins at boiling is a very convenient way of physico-chemical stabilisation without the need for extra filtration, which reduced beer losses and results in beers with an improved flavour stability.

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5 Reference

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Appendix

Table 1 First series of brewing trials on 50 L scale with mashing-in pH of 5.6

| Brewing experiment | Mashing-in conditions | | Concentration of gallotannins (g/hL) | Concentration of PVPP (g/hL) | Contact time (min) |
|--------------------|-----------------------|-----|--------------------------------------|------------------------------|--------------------|
| | Temperature (°C) | pH | | | |
| A | 63 | 5.6 | – | – | – |
| B | 63 | 5.6 | 5 | – | 3 |
| C | 63 | 5.6 | 10 | – | 3 |
| D | 63 | 5.6 | – | 10 | 3 |
| E | 63 | 5.6 | – | 10 | 5 |
| F | 63 | 5.6 | 10 | 10 | 6/5 |

Table 2 Second series of brewing trials on 50 L scale with mashing-in pH of 5.2

| Brewing experiment | Mashing-in conditions | | Concentration of gallotannins (g/hL) | Concentration of PVPP (g/hL) | Contact time (min) |
|--------------------|-----------------------|-----|--------------------------------------|------------------------------|--------------------|
| | Temperature (°C) | pH | | | |
| G | 63 | 5.2 | – | – | – |
| H | 63 | 5.2 | 5 | – | 3 |
| I | 63 | 5.2 | 10 | – | 3 |
| J | 63 | 5.2 | 10 | 10 | 6/5 |

Table 3 Standard analyses of the beers brewed on 50 L scale (first series)

| | A | B | C | D | E | F |
|--------------------------------------|-------|------------|------------|--------------|--------------|------------------------|
| pH at mashing in | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 |
| Application | – | 5 g/hL GT* | 10 g/hL GT | 10 g/hL PVPP | 10 g/hL PVPP | 10 g/hL of GT and PVPP |
| Contact time | – | 3 min. | 3 min. | 3 min. | 5 min. | 6 min./5 min.** |
| Alcohol (% v/v) | 5.14 | 4.98 | 4.73 | 4.65 | 4.77 | 5.20 |
| Real extract (% m/m) | 4.22 | 4.29 | 4.06 | 3.95 | 4.12 | 4.11 |
| Apparent extract (% m/m) | 2.36 | 2.49 | 2.34 | 2.25 | 2.39 | 2.23 |
| Apparent extract (g/100 ml) | 2.38 | 2.51 | 2.35 | 2.27 | 2.40 | 2.24 |
| Original gravity (°P) | 12.03 | 11.86 | 11.27 | 11.04 | 11.39 | 12.01 |
| pH | 4.47 | 4.45 | 4.44 | 4.22 | 4.25 | 4.31 |
| Colour (EBC) | 9.0 | 6.0 | 5.9 | 5.8 | 6.1 | 7.6 |
| Chill haze (EBC) | 0.20 | 0.32 | 0.30 | 0.56 | 0.60 | 0.66 |
| Permanent haze (EBC) | 0.77 | 0.26 | 0.22 | 0.67 | 0.64 | 0.99 |
| Sensitive Proteins (EBC) | 5.94 | 2.76 | 1.76 | 4.84 | 4.70 | 2.67 |
| Total polyphenols (mg/L) | 142.7 | 182.0 | 223.0 | 93.5 | 100.0 | 199.3 |
| Flavanoids ((+)- catechin eq.; mg/L) | 32.7 | 31.0 | 31.7 | 22.4 | 24.0 | 25.3 |
| Proanthocyanidins (mg/L) | 23.0 | 26.6 | 27.3 | 13.9 | 15.9 | 18.9 |
| TRAP (Asc. Acid. eq.; mmol/L) | 1.091 | 1.221 | 1.337 | 0.772 | 0.796 | 1.396 |
| DPPH (ΔA 10 min) | 0.795 | 1.037 | 1.218 | 0.635 | 0.702 | 1.227 |

*GT = Gallotannins

** There was one minute interval between the two applications.

Table 4 Standard analysis on beers stabilised with gallotannins and with pH 5.2 at mashing in (second series)

| | G | H | I | J |
|-------------------------------------|----------|-----------|------------|------------------------|
| pH at mashing in | 5.2 | 5.2 | 5.2 | 5.2 |
| Application | – | 5 g/hL GT | 10 g/hL GT | 10 g/hL of GT and PVPP |
| Contact time | – | 3 min. | 3 min. | 6 min/5 min. |
| Alcohol (% v/v) | 4.84 | 4.86 | 4.84 | 4.75 |
| Real extract (% m/m) | 4.09 | 4.04 | 4.41 | 4.13 |
| Apparent extract (% m/m) | 2.33 | 2.28 | 2.66 | 2.40 |
| Apparent extract (g/100ml) | 2.34 | 2.29 | 2.68 | 2.42 |
| Original gravity (°P) | 11.46 | 11.45 | 11.76 | 11.37 |
| pH | 4.38 | 4.28 | 4.32 | 4.27 |
| Colour (EBC) | 6.3 | 7.1 | 6.8 | 6.3 |
| Chill haze (EBC) | 0.39 | 0.50 | 0.75 | 0.68 |
| Permanent haze (EBC) | 0.34 | 0.75 | 0.65 | 0.71 |
| Sensitive Proteins (EBC) | 8.58 | 4.36 | 3.27 | 3.00 |
| Total polyphenols (mg/L) | 118.1 | 163.4 | 195.6 | 153.6 |
| Flavanoids ((+)-catechin eq.; mg/L) | 22.3 | 23.5 | 24.8 | 18.8 |
| Proanthocyanidins (mg/L) | 17.8 | 19.4 | 18.5 | 16.9 |
| TRAP (Asc. Acid. eq.; mmol/L) | 0.959 | 1.279 | 1.446 | 1.168 |
| DPPH (ΔA 10 min) | 0.828 | 0.973 | 1.226 | 0.953 |

Table 5 Content of gallic acid in the fresh beers

| Beer | g/hL | | Contact time (minutes) | pH at mashing in | Concentration Gallic acid (ppm) |
|------|--------------|------|---------------------------|---------------------|------------------------------------|
| | Gallotannins | PVPP | | | |
| A | – | – | – | 5.6 | 0.98 |
| B | 5 | – | 3 | 5.6 | 3.10 |
| C | 10 | – | 3 | 5.6 | 6.70 |
| D | – | 10 | 3 | 5.6 | 0.49 |
| E | – | 10 | 5 | 5.6 | 0.48 |
| F | 10 | 10 | 6/5 | 5.6 | – |
| G | – | – | – | 5.2 | 1.30 |
| H | 5 | – | 3 | 5.2 | 2.93 |
| I | 10 | – | 3 | 5.2 | 5.33 |
| J | 10 | 10 | 6/5 | 5.2 | – |

Table 6 Sensory evaluation of the fresh and aged beers (forced ageing at 40 °C)

| Beer | Ageing scores (0, 5, 10 and 15 days at 40 °C) | | | |
|------|---|---|----|----|
| | 0 | 5 | 10 | 15 |
| A | 0 | 4 | 6 | 7 |
| B | 0 | 3 | 5 | 6 |
| C | 0 | 3 | 5 | 7 |
| D | 0 | 5 | 6 | 8 |
| E | 0 | 4 | 5 | 7 |
| G | 0 | 4 | 6 | 7 |
| H | 0 | 5 | 5 | 6 |
| I | 0 | 4 | – | 6 |
| J | 0 | 4 | 5 | 7 |

A scale of 0–8 was used for determination of the degree of staling (0: fresh; 2: very slightly stale; 4: slightly stale; 6: strongly stale; 8: very strongly stale, undrinkable)

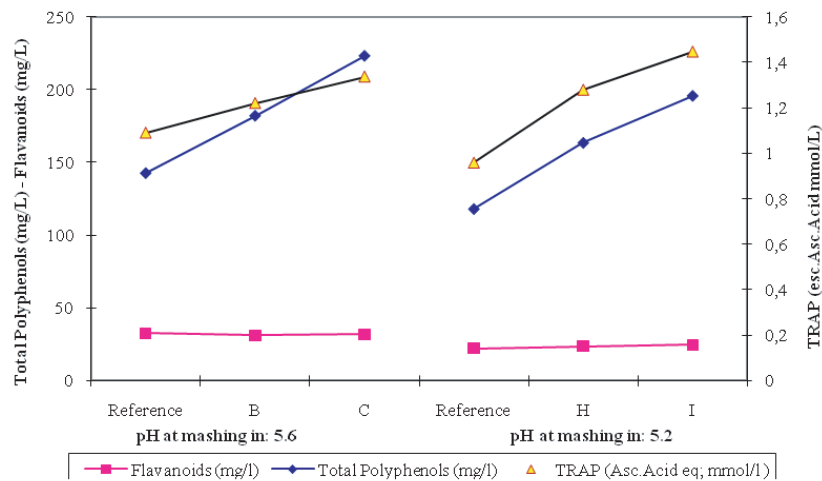


Fig. 1 Reducing power measured with the TRAP – assay [5] and polyphenols in finished beers treated with gallotan-

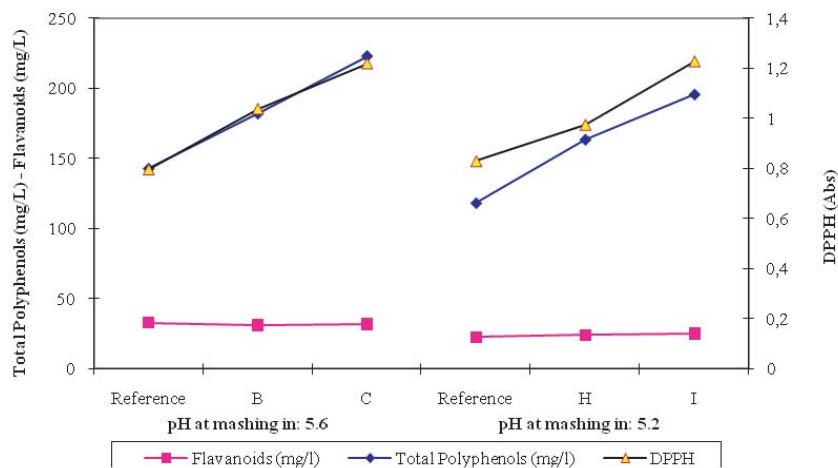


Fig. 2 Reducing power measured with the DPPH – test [9] and polyphenols in finished beers treated with gallotannis

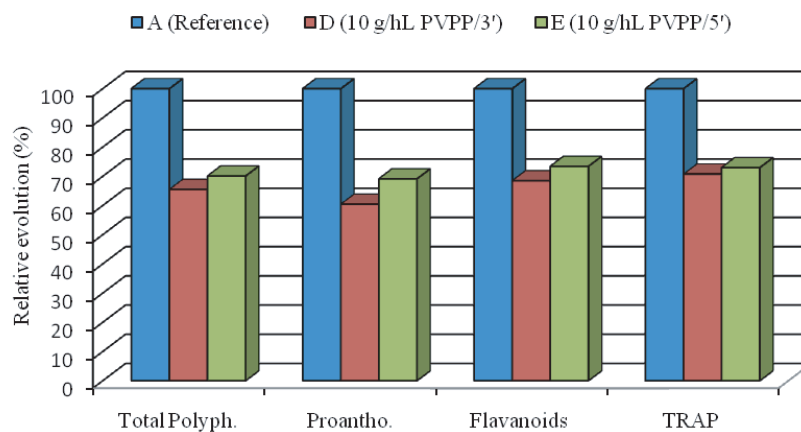


Fig. 3 Effect of PVPP treatment at the end of wort boiling on the amounts of polyphenols in finished beer

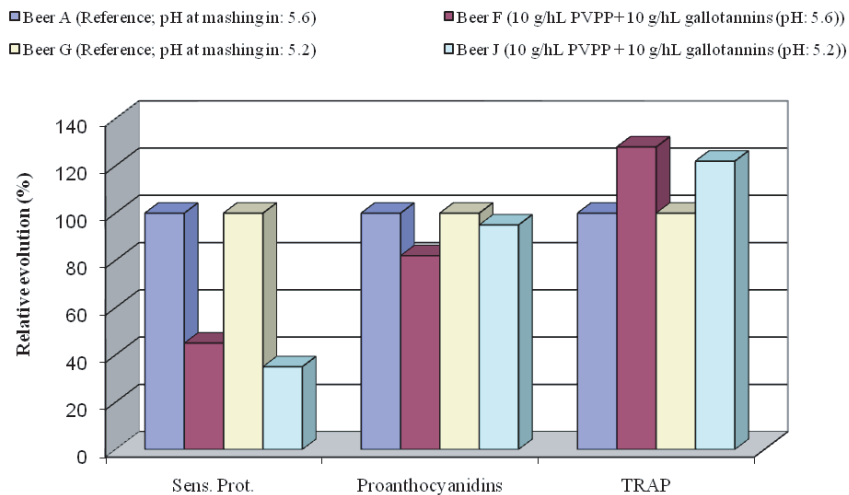


Fig. 4 The effect of addition of both gallotannins and PVPP on the amount of sensitive proteins and the reducing power (TRAP and DPPH)

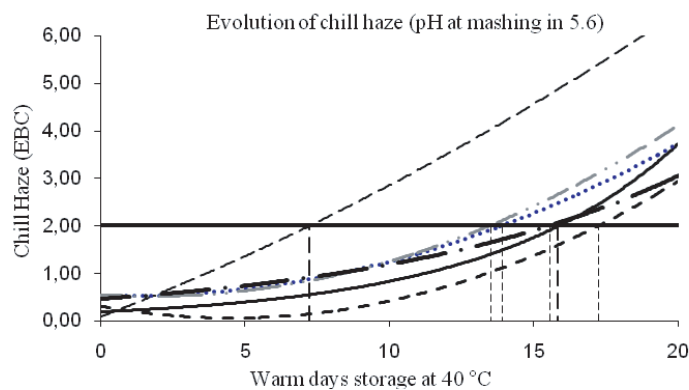


Fig. 5 The evolution of chill haze development in the first series of beers upon warm storage (40 °C)
 - - - = Beer A; - - - = Beer B (gallotannins: 5 g/hL; 3');
 - = Beer C (gallotannins: 10 g/hL; 3'); ... = Beer D (PVPP: 10 g/hL; 3');
 - = Beer E (PVPP: 10 g/hL; 5'); - = Beer F (10 g/hL gallotannins and 10 g/hL PVPP; 5')
 The horizontal line represents a haze of 2 EBC units

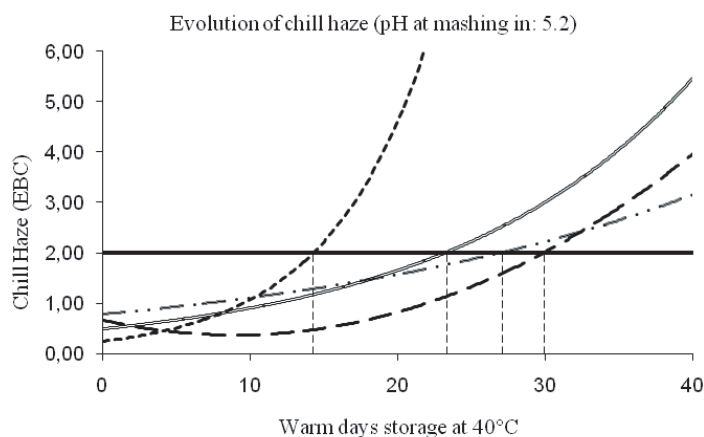


Fig. 6 The evolution of chill haze development in the second series of beers upon warm storage (40 °C)
 - - - = Beer G; = = Beer H (gallotannins: 5 g/hL; 3');
 - = Beer I (gallotannins: 10 g/hL; 3'); - - - = Beer J (10 g/hL gallotannins and 10 g/hL PVPP; 5')
 The horizontal line represents a haze of 2 EBC units