# The Loss of Hydrophobic Polypeptides during Fermentation and Conditioning of High Gravity and Low Gravity Brewed Beer

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ABSTRACT

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The object of this study was to investigate the loss of hydrophobic polypeptides, which are important for foam quality and stability in finished beer. Loss of hydrophobic polypeptide due to fermenter foaming occurs during transfer of fermented wort since a gradient of hydrophobic polypeptides towards the surface is created during fermentation. Due to higher polyphenol levels in high gravity (20°Plato) wort, more hydrophobic polypeptides are lost due to cold break (cold trub) precipitation compared to low gravity (12°Plato) wort. Another important factor affecting the loss of hydrophobic polypeptides could be proteinase A activity during fermentation, especially in high gravity fermentation where the yeast is exposed the higher stress. During high gravity fermentation, where osmotic pressures are higher, ethanol levels become greater, and nitrogen-carbohydrate ratios are lower, more proteinase A is released by the yeast. This release of proteinase A into fermenting wort could have implications for the foam stability of the finished product.

**Key words:** Cold break, foam stability, high gravity brewing, hydrophobic polypeptides, proteinase A.

## INTRODUCTION

A problem that still exists is that beer brewed at high gravities has poor head stability[4,8]. It has been found that the high gravity brewed beers have less hydrophobic polypeptides than low gravity brewed beer when measured at the same alcohol concentration[5]. It has been reported that this problem originates from poor extraction of hydrophobic polypeptides during mashing. The reason could be that during mashing a saturation point is reached in the wort and therefore no further hydrophobic polypeptides can be extracted into wort. The main points of loss of hydrophobic polypeptides occur during kettle boil and fermentation. Cooper et al.[5] measured hydrophobic polypeptides throughout fermentation and found that the high gravity wort (20°Plato) lost 47% and the low gravity wort (10°Plato) lost just 18% of the initial amount of hydrophobic polypeptides at the start of fermentation. Therefore, if the foam formation and stability of high gravity brewed beer is to be improved, it will be necessary to improve hydrophobic polypeptide extraction during mashing and/or minimise their losses during later stages of the brewing process. The fermentation stage is a key step where hydrophobic polypeptides are lost during the brewing process[21] (Fig. 1).

Three factors could account for the loss of hydrophobic polypeptides during fermentation and conditioning and these have been investigated. First, losses could be due to adhesion of the hydrophobic polypeptides onto the side of the fermentation vessel during transfer of fermenter wort to the conditioning vessel and due to adsorption onto the surface of the yeast cells. Second, foam-positive hydrophobic polypeptides could be lost due to the precipitation of cold break (cold trub) in the first days of fermentation. Finally, proteolytic activity, especially yeast proteinase A which is released by the yeast during fermentation could reduce hydrophobic polypeptide levels by degrading them[6].

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## MATERIALS AND METHODS

### Wort production

All malt worts were produced in the range of 12°Plato to 20°Plato in the ICBD's 2-hectolitre brewery and were hopped with kettle pellets to obtain beer bitterness of 16 IBU after dilution to 4.5% (v/v) alcohol.

### Fermentation

12°Plato and 20°Plato worts were fermented using either of two lager or two ale strains. The wort $DO_2$ was adjusted to 1 mg/°Plato and the pitching rate was $10^6$ cells/ml per °Plato for both lager and ale yeasts. The fermentation temperature was kept constant at 12°C for lager yeast and 20°C for ale yeast. Conditioning temperature was 5°C for lager yeast and 9°C for ale yeast and in both cases the temperature was adjusted to –1.5°C two days prior to filtration. Conditioning started from day 5–7 for low gravity brews and from day 10–12 for high gravity brews.

### Analyses of hydrophobic polypeptides

Samples from fermentation and conditioning were analysed for hydrophobic polypeptide content using hydrophobic interaction columns (HIC)[2].

### Extracellular proteinase A activity

Measurement of yeast extracellular proteinase A activity was carried out using a fluorescence assay[13].

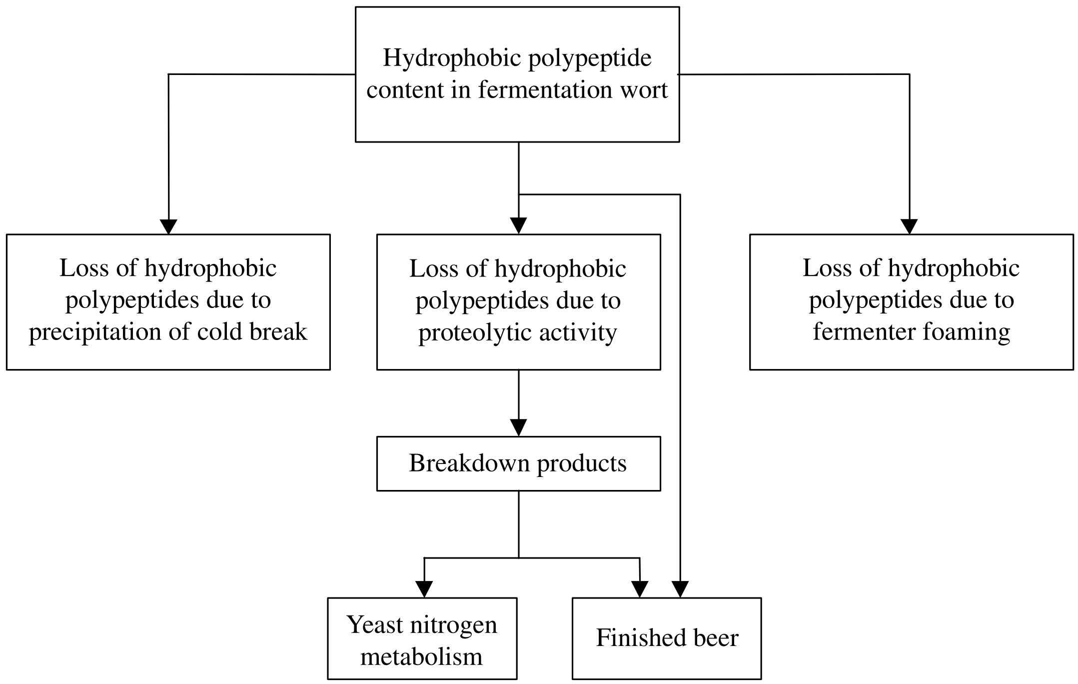



Fig. 1. The loss of hydrophobic polypeptides during fermentation and conditioning.

### Measurement of total polyphenols, total carbohydrates and proteins

Total polyphenols were determined using the IOB method[19], total carbohydrates according to the Analytica-EBC[1] and proteins using the Bradford method[3].

### Analysis of cold break

Measurement of the cold break weight was carried out using the method adapted from MEBAK[17]. The contribution of polyphenols, carbohydrates, protein and hydrophobic polypeptides to the cold break was determined by the difference before and after cold break precipitation.

### Determination of the cold break

All wort samples were collected before wort cooling and were analysed for cold break using the method by MEBAK[17]. The wort sample (200 ml) was adjusted to 20°C and filtered with a fluted filter (Elderol, Cat.-No. 21283). After cooling the elute to 5°C for 24 h, samples (50 ml) were membrane filtered in triplicate with an NC Filter (Whatman Black Grid, Cat.-No. 7153004) with a pore size of 0.45 µm. The filter paper was dried at 105°C for 1 h and then cooled in a desiccator for 30 min to ambient temperature. The dry weight of the material on the filters was determined and the average calculated.

## RESULTS AND DISCUSSION

### Losses of hydrophobic polypeptides due to fermenter foaming

During fermentation, foam active substances are progressively lost as a result of foaming inside the fermentation vessel induced by a large release of $CO_2$ from the yeast[11]. Therefore the larger the amount of the fermentable extract in the fermenting wort, the greater is the amount of $CO_2$ produced by the yeast. This observation was con-

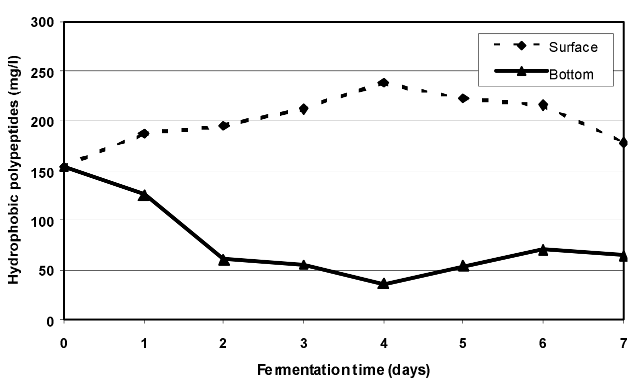



Fig. 2A. Stratification of hydrophobic polypeptides in a vessel during low gravity (12°Plato) fermentation.

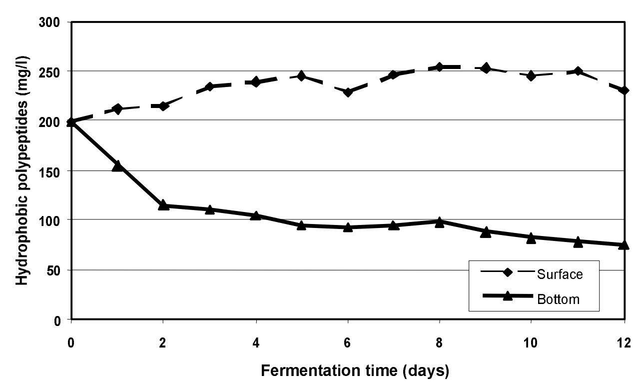



Fig. 2B. Stratification of hydrophobic polypeptides in a vessel during high gravity (20°Plato) fermentation.

firmed by Cooper et al.[5], who found that high gravity wort produces a greater amount of foam head than low gravity wort. Stewart et al.[21] also reported that high gravity wort fermentations had a more negative effect on beer hydrophobic polypeptides than low gravity fermentations. It is unlikely that the hydrophobic polypeptides are being degraded when they have left the liquid phase of the wort and moved into the foam phase. At the end of fermentation, when $CO_2$ production is reduced due to the lack of fermentable extract, the hydrophobic polypeptides should drop back in the liquid phase without any changes to their structure. However, it is believed that losses occur due to adhesion of the hydrophobic polypeptides onto the side of the fermentation vessel during transfer from the fermenter to the storage vessel[4,5].

During high and low gravity fermentation, the concentration of hydrophobic polypeptides at the bottom and just under the surface of the fermenter vessel was determined. It was observed that the concentration of hydrophobic polypeptides at the surface was approximately 4 times higher than at the bottom of the high gravity fermenter. This was also observed in the low gravity fermenter (Fig. 2 (A–B)).

In order to verify the stratification of hydrophobic polypeptides, and establish whether the higher concentration of hydrophobic polypeptides at the surface results from a general fluid mechanical property of the vessel, free amino nitrogen and polyphenols were also determined (Fig. 3 (A–D)).

No concentration gradient was observed for free amino nitrogen or polyphenols. This indicates that this phenomenon of a hydrophobic polypeptide gradient cannot be the result of a particular fluid mechanical property of the fermentation vessel. An explanation for the different concentration of hydrophobic polypeptide might be that the polypeptides are brought to the surface during $CO_2$ production of the yeast. The hydrophobic part of the polypeptide interacts with the gas phase of the $CO_2$ bubble and the gas bubble climbs to the surface. Once the polypeptide is at the surface it can interact on the $CO_2$/wort interface. The other components such as free amino nitrogen and polyphenols do not have the potential to interact with the gas phase of the $CO_2$ bubble and therefore no stratification of these parameters occurs.

Stassi et al.[20] suggested that foam formation during fermentation is a function of the foamability of the wort, the stability of the wort foam and the $CO_2$ evolution rate (CER) during fermentation. While the foamability and the foam stability factors are for the most part fixed by the brewhouse materials and procedures, and often cannot be varied, the CER may be controlled by many different factors. Stassi et al.[20] chose to modify the fermentation temperature profile and reduced successfully the maximum foam height during fermentation without producing a deleterious effect on product quality and/or total fermentation time.

The fermenter foam on its own has no direct influence on the loss of hydrophobic polypeptide. The losses occur

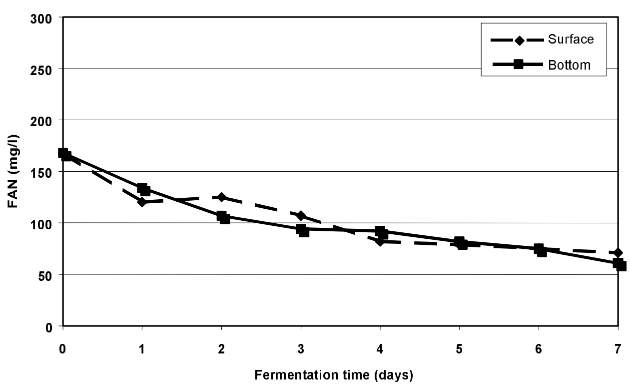



Fig. 3A. Stratification of free amino nitrogen in a vessel during low gravity (12°Plato) fermentation.

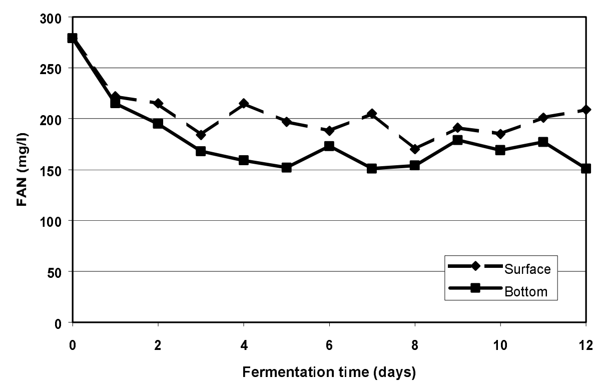



Fig. 3B. Stratification of free amino nitrogen in a vessel during high gravity (20°Plato) fermentation.

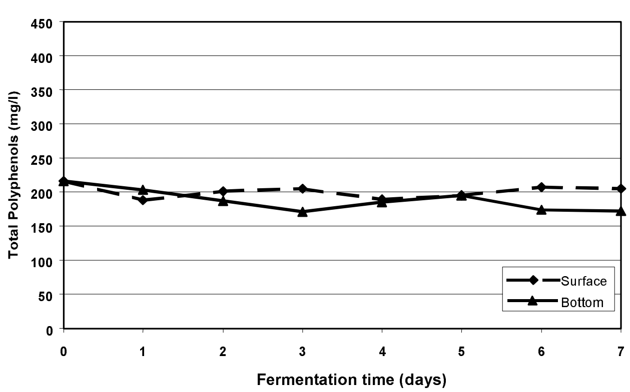



Fig. 3C. Stratification of polyphenols in a vessel during low gravity (12°Plato) fermentation.

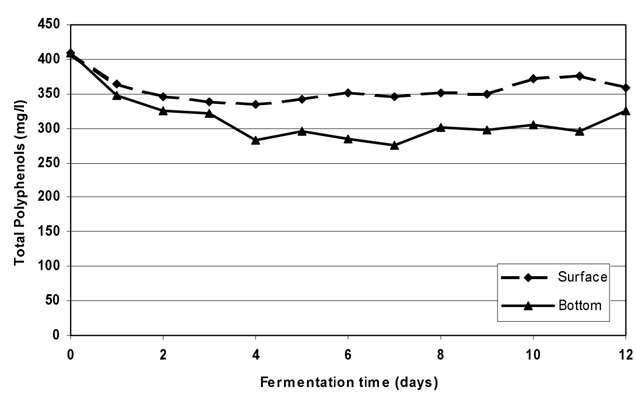



Fig. 3D. Stratification of polyphenols in a vessel during high gravity (20°Plato) fermentation.

when the fermenting wort is transferred to the conditioning vessel. Due to the concentration gradient, higher amounts of hydrophobic polypeptides are near to the surface. When the wort is transferred from the bottom of the fermentation vessel to the conditioning vessel, foam with high concentrations of hydrophobic polypeptides will adhere onto the surface of the fermenter vessel and are lost. These losses depend on the diameter and height of the fermenter, the CER, and other brewhouse procedures.

### Losses of hydrophobic polypeptides due to precipitation of cold break

Cold break precipitation occurs when wort is cooled to the required pitching temperature after wort boiling. Once the wort is cooled, the cold break progressively precipitates. At approximately 60°C, wort starts to become turbid. This turbidity is due to small particles about 0.5 µm in diameter. Since the particles are small, cold break settles only slowly. The particles have the property of adhering to other particles, e.g. yeast cells or gas bubbles. When they adhere to yeast cells, they decrease the yeast contact surface and thereby reduce the fermentation rate. This is referred to as 'coating' the yeast[14]. About 150–350 mg/L cold break precipitates in a 12°Plato all malt wort. The cold break consists of about 50% protein, 15–25% polyphenols and 20–30% high molecular carbohydrates[9].

The main part of the cold break consists of protein-polyphenol compounds, which precipitate to a greater extent in relatively cold media and partially dissolve again on warming. The principal wort components involved in cold break formation are malt polypeptides, and proanthocyanidins derived from both malt and hops[7]. Therefore the total amount of precipitated cold break material in the fermenting wort is a function of the total protein and polyphenol concentration in the fermenting wort prior to yeast pitching and the temperature profile during fermentation. Hence, the loss of hydrophobic polypeptide should logically follow the same pattern as the cold break precipitation because the hydrophobic polypeptide fraction is, by definition, a part of the total protein and due to its hydrophobic property this fraction can create complexes with polyphenols.

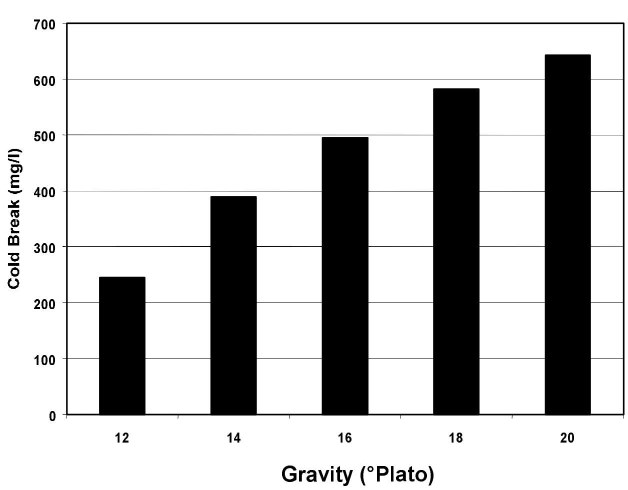



Fig. 4. The effect of wort gravity on cold break precipitation. All malt worts were prepared as described in the materials and methods. Sweet wort was cooled from 20°C to 4°C, left overnight and the precipitated material was then determined.

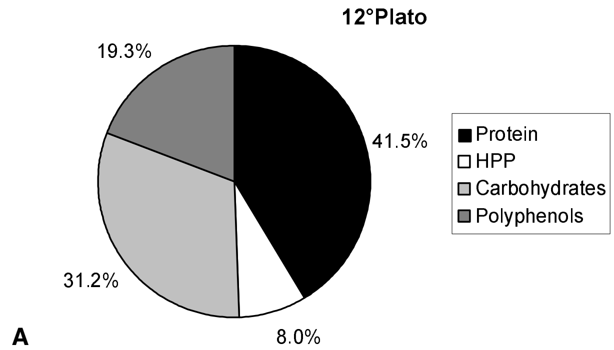
In order to investigate the effect of gravity on cold break precipitation, sweet wort was cooled from 20°C to 4°C, left overnight and the precipitated material was determined (Fig. 4).

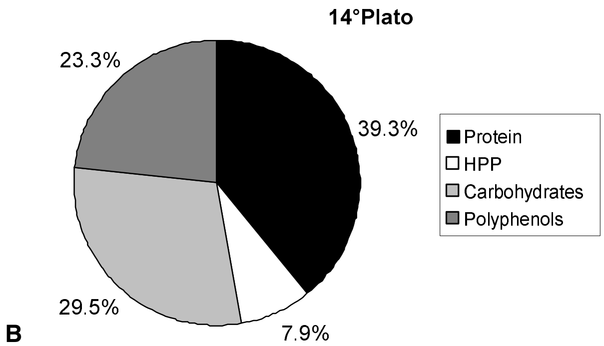
It can be observed that cold break does not precipitate in proportion to the increasing gravity of the wort. The gravity increased by 67%, from 12°Plato to 20°Plato whereas the total precipitated material increased from 245 mg/L to 643 mg/L approximately 162%. Table I shows the effect of increasing gravity on polyphenol and hydrophobic polypeptide levels in sweet wort and after cold break precipitation. As the gravity increases from 12°Plato to 20°Plato the sweet wort hydrophobic polypeptide content increased by 45% and the polyphenol content by about 75%.

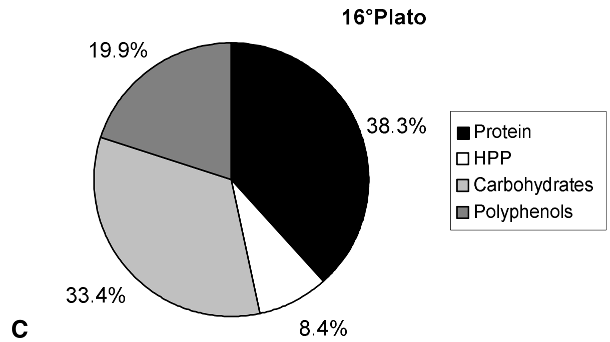
A reason why hydrophobic polypeptides are poorly extracted during mashing might be due to the temperature rise. Lewis et al.[15] showed that the dissolved protein decreased with rising mash temperature. In contrast, total polyphenols increased with higher mash temperature because of the more efficient extraction of polyphenols at higher temperatures. At the higher mashing temperatures two reactions take place. First precipitation of proteins with heat as a catalyst and second precipitation of polyphenol-protein complexes. These factors, in combination with the addition of polyphenols from hops during wort boiling, might be an explanation for the apparently poor extraction of hydrophobic polypeptides in comparison to polyphenols. Since the extraction of α-acids decreases as the gravity rises, a greater amount of hop is necessary in high gravity brews in order to achieve the same bitterness after dilution to the same alcohol level, compared with low gravity brews[18]. It can be observed that higher polyphenol levels, as well as higher polypeptide levels, remain in the wort after cold break precipitation (Table I). It has to be remembered that the finished beer from high gravity worts will be diluted to the same alcohol level as the finished beer from low gravity brewed wort. The higher polyphenol levels will not be a problem for the beer physical stability after dilution but the lower concentration of hydrophobic polypeptides is thought to be responsible for the poorer foam. Fig. 5 shows the contribution of protein, hydrophobic polypeptide, carbohydrates and polyphenols to cold break in different gravity worts. The contribution of the different fractions of the cold break was similar. Hence the polyphenols are binding to hydrophobic polypeptides in

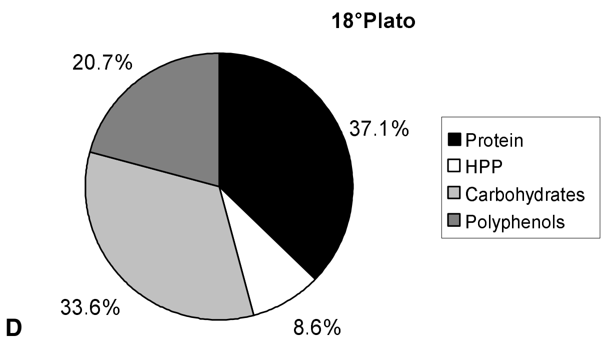
TABLE I: The effect of wort gravity on polyphenol and hydrophobic polypeptide levels in sweet wort before and after cold break precipitation. Values are means of triplicate determinations ± SDs.

| | Polyphenol (mg/L) | | Hydrophobic polypeptide (mg/L) | |
|---|---|---|---|---|
| Gravity (°P) | Sweet wort | After cold break precipitation | Sweet wort | After cold break precipitation |
| 12 | 216 ± 5 | 169 ± 6 | 162 ± 5 | 142 ± 5 |
| 14 | 252 ± 6 | 161 ± 5 | 181 ± 7 | 150 ± 6 |
| 16 | 279 ± 6 | 180 ± 9 | 205 ± 6 | 163 ± 5 |
| 18 | 324 ± 8 | 203 ± 7 | 223 ± 6 | 173 ± 6 |
| 20 | 378 ± 8 | 231 ± 6 | 235 ± 7 | 177 ± 5 |



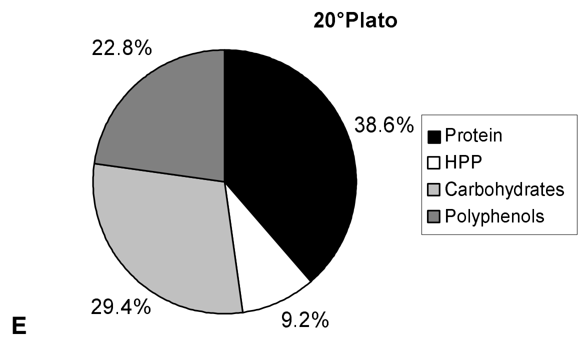




Fig. 5 (A–E). The effect of gravity on cold break composition. All malt worts were prepared as described in materials and methods.

the same weight ratio but not to the same extent. Thus, the higher amount of cold break precipitation can simply be explained by the increasing concentration of hydrophobic polypeptides and polyphenols with increasing gravities.

It would be expected that the cold break weight would increase to the same extent as hydrophobic polypeptide fraction because the hydrophobic polypeptide fraction is more poorly extracted than the polyphenol fraction. The difference in the determined weight of cold break as gravity increases must be due to a higher molecular weight of at least one of the partners responsible for the cold break precipitation. An explanation would be a higher affinity of polymerised polyphenols to hydrophobic polypeptides or by higher molecular carbohydrates associated with polyphenols and/or proteins.

In order to determine the loss of hydrophobic polypeptide which occurs due to the cold break precipitation, the following experiment was carried out. Cold break was allowed to precipitate in 12°Plato and 20°Plato wort with an adjusted pH of 5.3 and at a temperature of 12°C without yeast. This ensures that proteolytic activity from yeast cells could have no influence on the losses. The hydrophobic polypeptides were monitored over the time period of fermentation. Since there is no fermentation activity in the wort, the only possible source of hydrophobic polypeptide loss is due to the precipitation of cold break rather than proteolytic activity or fermenter foaming. Therefore it was possible to separate the losses due to cold break from those of other parameters. A control was carried out in which the wort was fermented as described in the materials and methods and compared with the wort in the absence of yeast.

The hydrophobic polypeptide levels were monitored during the length of a typical fermentation of either 12°Plato or 20°Plato wort. A similar trend as was observed during fermentation but to a different extent. Fig. 6 shows the different losses of hydrophobic polypeptides both for high gravity fermentation (20°Plato) and for cold break precipitation from 20°Plato sweet wort, respectively.

The total losses of hydrophobic polypeptides due to cold break precipitation in high gravity wort were 56% of the initial amount after 12 days in the sweet wort. As ex-

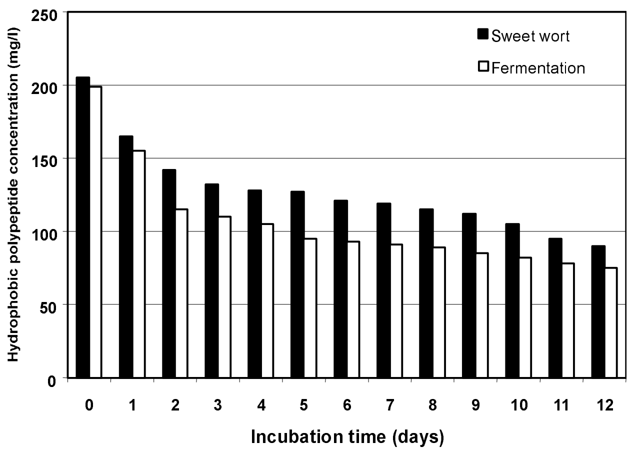



Fig. 6. The losses of hydrophobic polypeptides in sweet wort (20°Plato) adjusted to pH 5.3 without yeast and from high gravity fermentation (20°Plato). All malt wort was prepared as described in materials and methods. Values are means of triplicate assays.

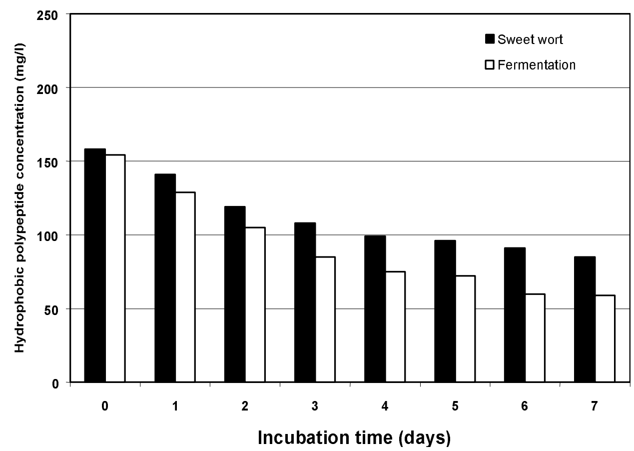



Fig. 7. The losses of hydrophobic polypeptides in sweet wort (12°Plato) adjusted to pH 5.3 without yeast and from low gravity fermentation (12°Plato). All malt wort was prepared as described in materials and methods. Values are means of triplicate assays.

pected the losses during fermentation were higher with 62% within the 12 days. The reason for this difference is thought to be due to proteolytic activity especially proteinase A which is secreted both by living yeast cells and released by dead yeast cells upon autolysis. In case of the 12°Plato fermentation the total losses of hydrophobic polypeptides were, after 7 days, 46% of the initial amount due to cold break precipitation and 61% during fermentation, and followed a similar pattern in 20°Plato wort (Fig. 7).

In order to assess the losses of hydrophobic polypeptides in the absence of fermentation, it was also necessary to investigate the influence of the pH decrease on the cold break precipitation during fermentation. Therefore, cold break was allowed to precipitate in two samples of sweet wort over a time period of 5 days at 12°C. The time period of 5 days was chosen because during fermentation the pH drops from the initial value to its final value within 5 days. Fig. 8A shows the hydrophobic polypeptide concentration in two samples of 20°Plato sweet wort. In the first sample the pH was adjusted to 5.3 whereas in the second sample the pH was artificially decreased with hydrochloric acid in the same way that can be observed during fermentation. At a constant pH of 5.3 the hydrophobic polypeptide level decreased from the initial amount of 215 mg/L to 105 mg/L at the 5th day, a loss of 51% of the initial hydrophobic polypeptide level. In the sample where the pH was artificially changed the hydrophobic polypeptide level decreased from the initial level of 210 mg/L to 108 mg/L at the end, a loss of 49%.

For the 12°Plato wort the same trend was observed (Fig. 8B). The sweet wort with a constant pH of 5.3 lost 36% of the initial hydrophobic polypeptides and the sweet wort, with an artificial pH decrease, lost 34% of the hydrophobic polypeptide.

These results show that the change of the pH during fermentation has little, if any, influence on cold break precipitation.

## Losses of hydrophobic polypeptide due to proteolytic activity

Another important factor for the losses of hydrophobic polypeptides is proteolytic activity during fermentation. Proteinase A is, amongst other proteases in fermenting

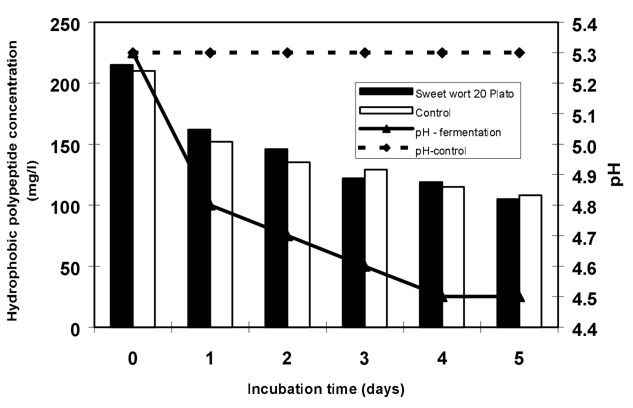



Fig. 8A. The dependence of cold break precipitation on the pH in high gravity (20°Plato) wort. Worts were prepared as described in Fig. 6.

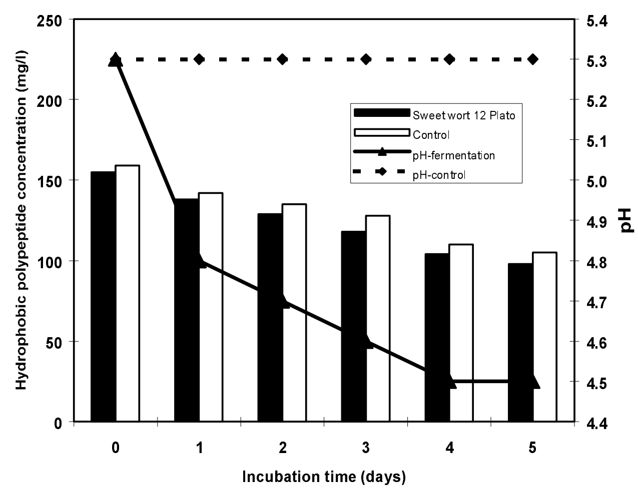



Fig. 8B. The dependence of cold break precipitation on the pH in low gravity wort (12°Plato). Worts were prepared as described in Fig. 7.

TABLE II: Proteinase A activity in all malt worts of high (20°Plato) and low (12°Plato) gravity at the start of fermentation and after conditioning. For experimental details see materials and methods. Values are means of triplicate determinations ± SDs.

| Yeast | 12° Plato Start fermentation (mU/mL) | 12° Plato End conditioning (mU/mL) | 20° Plato Start fermentation (mU/mL) | 20° Plato End conditioning (mU/mL) |
|---|---|---|---|---|
| Lager 1 | 0·213 ± 0·010 | 0·375 ± 0·012 | 0·374 ± 0·018 | 0·795 ± 0·030 |
| Lager 2 | 0·608 ± 0·029 | 0·764 ± 0·029 | 1·063 ± 0·031 | 1·365 ± 0·036 |
| Ale 1 | 0·246 ± 0·011 | 0·451 ± 0·026 | 0·447 ± 0·027 | 0·809 ± 0·028 |
| Ale 2 | 0·445 ± 0·023 | 0·557 ± 0·026 | 0·875 ± 0·029 | 1·085 ± 0·033 |

wort or beer, the most important because it has the highest activity for degrading foam active proteins at pH 4.0–4.5[12] and has a preference for cleaving peptide bonds linking, mostly large, hydrophobic amino acid domains[10]. Furthermore, proteinase A is an endoproteinase and hence only a low concentration of proteinase A is necessary to have an influence on foam stability of finished beer, because very few amino acid cleavages could have a significant effect on the size of a hydrophobic polypeptide and its hydrophobicity. Experiments were carried out on 20°Plato and 12°Plato worts to determine the influence of proteinase A, released by different yeast strains, on the loss of hydrophobic polypeptide levels during fermentation and conditioning. 20°Plato and 12°Plato all malt worts were fermented separately by four different yeasts, two lager and two ale yeast strains. Table II shows the proteinase A levels at start of fermentation and at the end of conditioning with the different yeast strains in high and low gravity wort fermentations.

The results show that fermenting wort contains proteinase A activity shortly after yeast pitching. This could be related to the proteinase A content in the yeast pitching slurry. This means that the initial concentration of proteinase A in fermenting wort depends on the proteinase A concentration in the yeast slurry and is related to the yeast storage procedures employed. For both 20°Plato and 12° Plato wort fermentation, the same yeast slurry was used and therefore the higher initial concentration in higher gravity wort can only be related to the pitching rate. The concentration of proteinase A in the yeast slurry is thought to be a function of yeast viability, vitality, the average age of individual yeast cells and the yeast storage conditions. In general, the increase of proteinase A activity in fermenting wort was greater in 20°Plato wort than in 12° Plato wort. This can be related to the higher osmotic pressure and higher alcohol levels in high gravity fermentations compared with low gravity fermentation, since no correlation was found between the viability of the yeast and the released proteinase A. Furthermore, the extent of proteinase A increase depended on the yeast strain.

Figs. 9 and 10 show the level of hydrophobic polypeptide throughout fermentation and conditioning conducted with two lager yeast strains in high (20°Plato) and low (12°Plato) gravity worts. In case of lager yeast one, the initial hydrophobic polypeptide concentration was 199 mg/L in 20°Plato wort. During fermentation 144 mg/L or 72% of the initial amount of hydrophobic polypeptides were lost. A similar trend was observed during the fermentation with lager yeast two. From the initial hydrophobic polypeptide concentration of 207 mg/L the wort lost 146 mg/L or 71% throughout fermentation and conditioning. The loss of hydrophobic polypeptides during low gravity fermentations showed a similar pattern. The unfermented 12°Plato wort contained 154 mg/L for the lager yeast one and 161 mg/L for the lager yeast two. The absolute amount of hydrophobic polypeptide at the end of fermentation was 57 mg/L for lager yeast one and 68 mg/L for lager yeast two and the losses were therefore 63% and 65%, respectively. That means that the losses in high gravity fermentation and conditioning were on average approximately 7% higher than in low gravity fermentation and conditioning.

Similar experiments were conducted with two ale yeast strains for 20°Plato and 12°Plato fermentation (Figs. 11 and 12). The initial levels of hydrophobic polypeptides in the fermentation worts were 193 mg/L for ale yeast one and 208 mg/L for ale yeast two, almost identical to the initial wort hydrophobic polypeptide concentration of the two lager yeast fermentations. The losses during fermentation were 142 mg/L or 73% for ale yeast one and 143 mg/L or 69% for ale yeast two, similar to the lager yeast fermentations. In case of the low gravity ale fermentations,

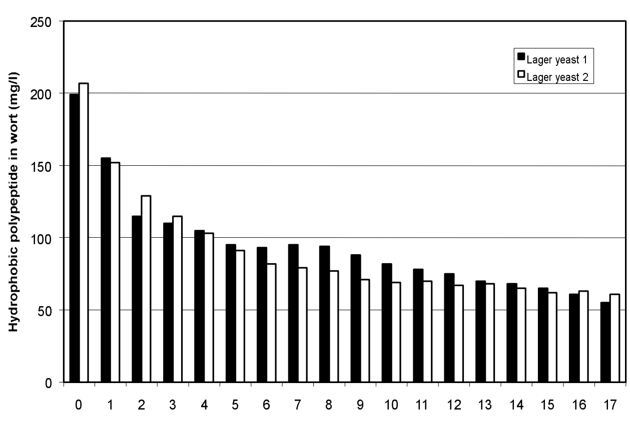



Fig. 9. The effect of all malt high gravity (20°Plato) wort on the losses of hydrophobic polypeptides during fermentation with lager yeast strains. Values are means of triplicate assays.

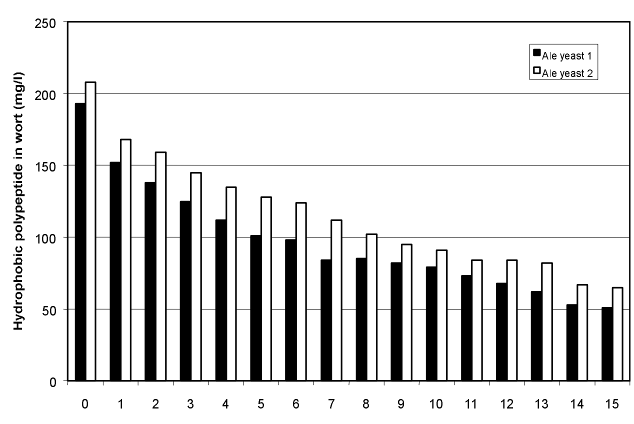



Fig. 10. The effect of all malt high gravity (20°Plato) wort on the losses of hydrophobic polypeptides during fermentation with ale yeast strains. Values are means of triplicate assays.

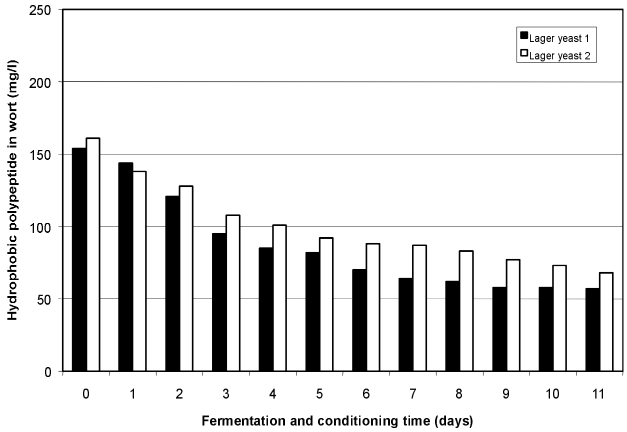



Fig. 11. The effect of all malt low gravity (12°Plato) wort on the losses of hydrophobic polypeptides during fermentation with lager yeast strains. Values are means of triplicate assays.

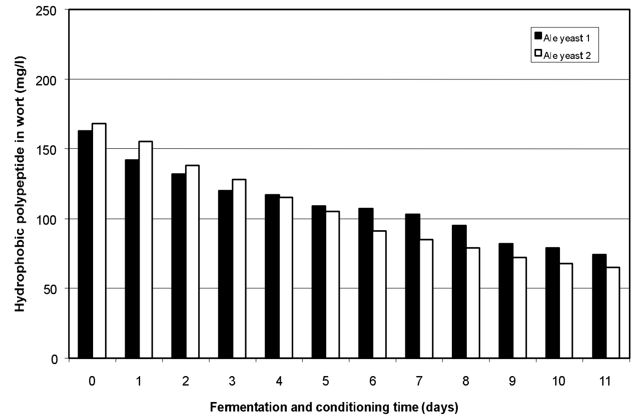



Fig. 12. The effect of all malt low gravity (12°Plato) wort on the losses of hydrophobic polypeptides during fermentation with ale yeast strains. Values are means of triplicate assays.

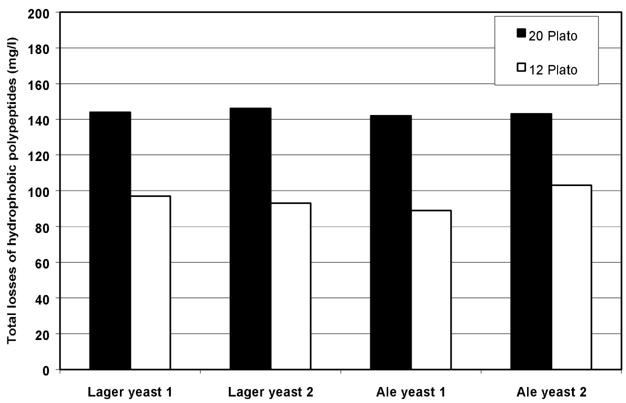



Fig. 13. Comparison of the losses of hydrophobic polypeptides during fermentation and conditioning with different yeast strains.

the same trend was observed as in the lager fermentations. The initial amount of hydrophobic polypeptide levels were 163 mg/L for ale yeast one and 168 mg/L for ale yeast two. The losses were 89 mg/L or 54% for ale yeast one and 103 mg/L or 61% for ale yeast two, similar to the lager yeasts. Fig. 13 shows a comparison of the losses of hydrophobic polypeptides during fermentation and conditioning for the four yeast strains at the two different fermentation gravities.

Although the initial amount of hydrophobic polypeptide was on average 25% greater in high gravity wort, the level of hydrophobic polypeptides at the end of high gravity fermentation was almost the same as in low gravity fermentation. However, it has to be remembered that once the high gravity wort has been diluted to the same alcohol level as the low gravity wort, it will contain a substantially lower hydrophobic polypeptide level. Hence, dilution of these polypeptides leads to poorer foam in the finished beer compared to the low gravity brewed beer. This result shows that even where levels of proteinase A differ in the fermenting wort, losses of hydrophobic polypeptides are apparently similar. The reason might be that the part of the hydrophobic polypeptides which are favoured by proteinase A have already precipitated with polyphenols, so that higher concentrations of proteinase A, which occur at the late stages of fermentation and during conditioning, have only a minimal effect on degrading the key hydrophobic polypeptides. Thus, it appears that the cold break precipitation limits the available substrate for proteinase A. Therefore an effect of higher proteinase A concentration on hydrophobic polypeptides cannot be observed during fermentation and conditioning but at the later stage of storage. Another reason might be that a part of the hydrophobic polypeptide fraction is proteinase A resistant. This would not be surprising since foam LTP, a significant part of wort hydrophobic polypeptides, is known to be proteinase resistant[16]. After dilution of high gravity brewed beer to the same alcohol level as low gravity brewed beer the proteinase A activity, as well as the hydrophobic polypeptide level, dropped according to the dilution factor. The ratio between hydrophobic polypeptide and proteinase A is of course the same after dilution. This higher ratio between proteinase A activity in high gravity brewed beer to the remaining hydrophobic polypeptide after dilution will have a negative effect on the head formation during storage time and this could be an important reason for poor foam stability in high gravity brewed beer.

### The ratio between proteinase A activity and hydrophobic polypeptide level in wort and its effect on foam stability during storage time

The effect of hydrophobic polypeptide content and proteinase A activity in the bottled beer on foam stability during storage time was investigated. High gravity (20°Plato) and low gravity (12°Plato) brewed beer was diluted to the same alcohol level (4.5% abv.) and stored over a time period of 3 months at 5°C. Figs. 14 and 15 show the decreasing foam stability in low gravity and high gravity beers, which were fermented by four different yeast strains.

It can be observed that the low gravity brewed beers have a higher Nibem value at the beginning of storage than their counterparts brewed as high gravity beer. This lower Nibem value stemmed from a lower initial hydrophobic polypeptide content in the bottled beer since a strong correlation ($R^2$ = 0.968) was found between hydrophobic polypeptide content in bottled beer and the Nibem value. The hydrophobic polypeptide concentration was similar in

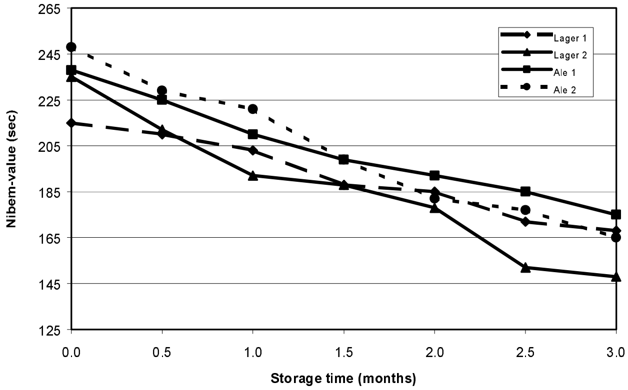



Fig. 14. The effect of storage time on foam stability in low gravity (12°Plato) brewed beer. Values are means of triplicate assays.

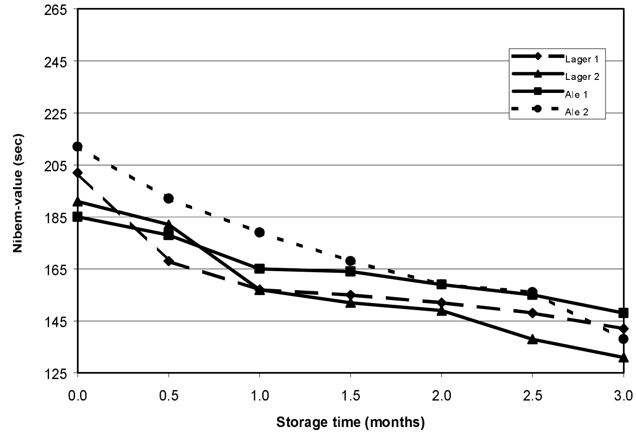



Fig. 15. The effect of storage time on foam stability in high gravity (20°Plato) brewed beer. Values are means of triplicate assays.

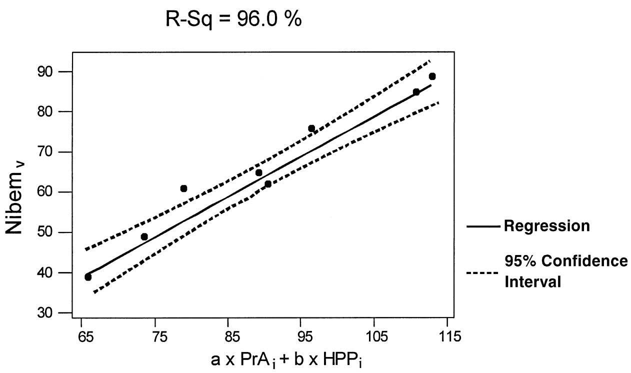



Fig. 16. Relation between the velocity of decrease of the Nibem value ($Nibem_v$) and the initial proteinase A activity ($Pr_i$) and the initial hydrophobic polypeptide content ($HPP_i$) in bottled beer during a storage time of 3 months.

both high and low gravity wort after conditioning. Once the beer was diluted to the same alcohol concentration, the hydrophobic polypeptide concentration was lower in high gravity brewed beer and hence the Nibem value decreased in proportion to the required dilution factor. In order to assess the decrease of foam stability over storage time at least two factors have to be considered. The initial Nibem value in the bottled beer and the velocity of decreasing foam stability. In this experiment, the velocity of decreasing foam stability ($Nibem_v$) was defined as the decrease of the Nibem value over a time period of 3 months. A strong relation was found ($R^2 = 0.960$) between the decrease in foam stability ($Nibem_v$), the hydrophobic polypeptide concentration and proteinase A activity ($PrA_i$) in the fresh bottled beer ($HPP_i$). Hence, the higher the initial amount of hydrophobic polypeptide and the higher the proteinase A activity in the fresh bottled beer the faster is the decrease of foam stability (Fig. 16).

In the case of pasteurised beer, where no proteinase A activity can be found, the velocity of decrease in foam stability would depend only on the initial amount of hydrophobic polypeptide, which would obviously not make sense. Furthermore, the apparent relationship between the velocity of decrease in foam stability and the initial amount of hydrophobic polypeptides implicates at least a further unknown factor with an important role in the decrease of foam stability, since this phenomenon can not be accounted for only by proteinase A activity. This unknown factor influences the foam stability in proportion to the initial Nibem value. This might be an enzymatic and/or chemical reaction, which takes place during storage.

## CONCLUSIONS

Loss of hydrophobic polypeptide due to fermenter foaming occurs only during transfer of fermentation wort from the fermentation vessel to the conditioning vessel. During fermentation, a gradient of hydrophobic polypeptides towards the surface is created. This gradient enhances adhesion of foam-active components onto the side of the fermentation vessel during the transfer to the conditioning vessel when the wort is usually pumped from the bottom of the fermentation vessel.

Almost certainly, a part of the hydrophobic polypeptides will interact with the cell wall of yeast due to adsorption but no significant decrease of hydrophobic polypeptide could be observed during yeast cropping. This suggests that the amount of adsorbed material does not play an important role in comparison to other sources of losses namely cold break precipitation and proteinase A activity.

Proteinase A is released by the yeast to a greater extent under high gravity fermentation conditions than under low gravity conditions. This increase of proteinase A activity depends on the yeast strain. This can depend on the different sensitivity of yeast strains to environmental stress.

Cold break precipitation is the dominant factor effecting hydrophobic polypeptide losses. This can be explained by a higher concentration of polyphenols and proteins in high gravity wort. Hence a lower concentration of preferred substrate is available for proteinase A. This means that following any technical efforts to reduce cold break formation, proteinase A will become a more dominant factor.

Therefore both sources of loss have to be considered simultaneously in order to assist hydrophobic polypeptides survive the fermentation and conditioning process. On the one hand more hydrophobic polypeptides would improve head stability but a higher concentration of proteins will probably decrease the colloidal stability of the finished beer.

Improvement could be achieved by reducing the polyphenol levels in the fermenting wort or by reducing the proteinase A activity in yeast slurry prior to pitching and/or reducing the proteinase A released during fermentation and conditioning. This will be a challenge in high gravity brewed beer with increased pitching rates, modified nitrogen/carbon ratios, and higher alcohol concentrations during fermentation and conditioning.

Furthermore, a strong correlation was found between hydrophobic polypeptide content and Nibem value in freshly bottled beer. A mathematical relationship was found between the velocity of the decrease of foam stability and the ratio between the initial amount of hydrophobic polypeptide and proteinase A activity in the bottled beer. This model implies that proteinase A is important but is not the only factor responsible for the foam stability decrease during storage.

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