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Yeast Quality Distribution in the Cone of Cylindro Conical Tanks

Yeast cropping from cylindro-conical tanks and repitching of the yeast is a key procedure in the brewing industry. In general it is assumed that the yeast collected from the cone of a tank is a homogenous culture. However results from studies investigating this aspect indicate that yeast quality as well as the environmental conditions within the cone can vary extensively. Results from different studies have been contradictory in various aspects. In order to add more information to this topic several yeast crops in two industrial breweries were followed obtaining samples in certain intervals. Each sample was analyzed for the physiological conditions of the yeast and various characteristics of the recoverable beer were investigated. In addition for some trials the replicative age and the fermentation performance in EBC Tall tubes have been examined. It was found that yeast vitality and viability did not vary considerably throughout the individual crops but large differences were found in-between several crops. Similar results were found for the characteristics of the recoverable beer. Regarding the replicative cell age a gradient throughout the crop was found. But the fermentation performance of the individual samples could not be related to the cell age.

Descriptors: yeast, cropping, viability, vitality, fermentation performance

1 Introduction

Cylindro-conical fermentation tanks are predominantly used for beer production worldwide. Besides several other advantages one important feature of cylindro-conical tanks is that the yeast sediments to the cone and can be cropped as a single phase prior the beer collection. For the use in the breweries it is assumed that the yeast quality throughout the cone is homogenous. But the little research which has been done on this topic indicates that certain yeast parameters vary throughout the cone. *Deans et al.* [1] found an accumulation of older cells at the bottom of the cone and that yeast collected at different points in the cone showed different fermentation performance. In contrast to these findings *Powell et al.* [2] found the highest concentrations of older cells in the middle of the cone. This study [2] found as well differences throughout the cone for the cell size and flocculation tendency. The barm beer surrounding the cell varied in specific gravity, alcohol content and pH. The findings of flocculation tendency of the cropped yeast as well as alcohol content and pH of the barm beer are supported by *Quain et al.* [3].

In terms of yeast quality Deans et al. [1] tested samples from different parts of the cone on their fermentation performance and found indications that the mixed aged cells from the middle of the cone had the best fermentation capacity. Powell et al. [2] as well as Quain et al. [3] measured the yeast viability of the cells throughout the cone and found great differences of up to 20 % (74 to 94 %) and 12 % (76 to 88 %) respectively. In both investigations only a minor trend toward a decrease in viability during the yeast cropping process (representing the different layers in the cone) was found. As another aspect of yeast quality Powell et al [2] analyzed the glycogen content of yeast from different layers and these data indicate that the cells towards the bottom of the cone are more depleted of this storage carbohydrate. Glycogen is often used as an indicator of yeast vitality since it plays an essential role as energy source for the lipid synthesis at the start of the fermentation when the uptake of wort sugars has not yet started. Therefore upper layers of yeast in the cone which are higher in glycogen would be considered healthier than the yeast near the bottom. However, the quality of glycogen as a vitality indicator is discussed controversially. Some researchers found a correlation between glycogen content and fermentation performance [4, 5, 6] while others where not able to confirm this relationship [7, 8].

In general it can be stated that the knowledge about the quality of the yeast in the cone and possible variations throughout the cone is very limited. This is somehow surprising since the yeast quality is a crucial factor for the fermentation in terms of consistency and efficiency as well as product quality. It is common knowledge that yeast during the storage in the cone is subject to various stress factors such as alcohol [2, 9], temperature [4, 6, 10] starvation [11] as well as osmotic [12] and hydrostatic pressure [13, 14]. These

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Tables and Figures see Appendix.

stresses can cause a deterioration of the physiological condition of yeast [4, 15].

To gain more information about the distribution of yeast quality within the cone several yeast crops from production-scale fermentations at two breweries have been investigated. Samples where taken throughout the cropping process representing different layers in the cone and the yeast vitality was analyzed using the intracellular pH measurement which had proven to be an reliable indicator for the physiological condition of yeast [16, 17, 18, 19]. As well the yeast viability and various characteristics of the barm beer were analyzed.

2 Material and Methods

2.1 Crop Sampling

Crop samples were optained from two different breweries. In case of brewery A the crop was collected from 3.000-hl fermentation vessel (dimensions 22.5 × 4.8 m; cone angle 65 °) and the total volume of cropped yeast was 80 to 90 hl. In case of brewery B from a 1.200 hl fermentation vessel (dimensions 14.5 × 3.24 m; cone angle 65 °) and the total volume of cropped yeast was 25 to 30 hl. In both cases samples were collected in certain intervals throughout the cropping process at a point close to the yeast collection vessel. Samples were stored immediately at 2 °C and were processed (analyzed + use for EBC tall tub fermentation) within 48 h.

In Brewery A cropping regime which is in literature referred to as warm cropping [2, 3, 20] was employed. The yeast crop was carried out at the latest 100 h after the last brew entered the tank. This means the yeast is recovered before the completion of the diacetyl reduction. In Brewery B the yeast was cropped after the diacetyl reduction and after a substantial cool down of the fermentation vessel (conventional cropping). Cropping time was approximately after 130 to 150 h.

2.2 Analysis of Barm Beer

The analysis of Extract, Alcohol, pH and FAN were performed according to procedures outlined in the recommended methods of the "Mitteleuropäische Brautechnische Analysenkommision" MEBAK [21].

2.3 Analysis of Cropped Yeast

Viability. Yeast viability was determined with the methylene blue staining method using a citrate methylene blue solution [22].

Vitality. Yeast vitality was determined using a modified version of the intracellular pH measurement (ICP) [23].

Cell Age Determination. The yeast age was determined enumerating the bud scars present on the cell wall according to a method of Deans et al. [1] with minor changes as outlined below. Yeast was harvested and washed three times in distilled water (centrifugation condition: 2000 x g for 3 min at 2 °C). Equal volumes of cell suspension and Calcofluor solution (1 mg mL-1, Sigma Chemical Co., St. Louis, MO/USA) were mixed and yeast was examined by fluorescence microscopy using U.V. excitation. The bud scars of at least 200 individual cells where enumerated and grouped into the following classes: 0 scars; 1-2 scars; 3-4 scars; 5-6 scars and more than 7 scars.

EBC Tall Tube Fermentations. With the yeast samples collected throughout the cropping process fermentations in tall tubes have been pitched in duplicate. All-malt worts were collected from an industrial scale Brewery (Bavarian State Brewery Weihenstephan) and the yeast was pitched into the aerated wort at a concentration of 12 Mio Cells/ml.

3 Results and Discussiom

3.1 Physiological Conditions of Yeast Within the Cone

In general in the breweries yeast storage time is considered the time from yeast crop until the repitching of the yeast. However the storage time commences already in the fermentation vessel when the yeast has settled to the bottom and has no longer access to nutrients. The physiological conditions of yeast is known to deteriorate over the duration of the storage [2, 4, 10, 11, 15]. In order to assess the yeast vitality a modified intracellular pH Measurement was used. The importance of the intracellular pH for the yeast physiology is based on several factors. For example the plasma membrane ATPase regulates the intracellular pH, which is essential for yeast growth [24]. The transmembrane H+ gradient as a result of the plasma membrane ATPase activity is a driving force for the uptake of nutrients [25, 26, 27]. Furthermore the intracellular pH regulates key enzymes in glycolysis and gluconeogenesis by the regulation of the c-AMP [28, 29]. For the actual measurement a low extracellular pH is applied (pH 3) and if the cells show high plasma membrane ATPase activity they are able to maintain a high intracellular pH indicating good yeast vitality (>6.2 = high vital; 5.8 to 6.2 = average to good vitality; <5.8 = poor vitality).

In trial 1 in Brewery A one yeast crop was followed (Fig. 1) and the results showed very high vitality values (approx. pH 6.6) and basically no variation throughout the cone was noticed.

To confirm these results in a second trial the crop of another fermentation vessel was followed (Fig. 2) showing as well high vitality values and hardly any variation throughout the cone. For the second trial as well the viability of the yeast samples was evaluated (Fig. 2) The results support the vitality observation. The overall viability was very high (arithmetic mean 98.0 %) with hardly any variation over all samples (± 0.5 % Least Significant Difference (LSD) Confidence Level 0.95). These results are comparable to the findings of Quain et al. [3] who found values of 91.1 ± 1.6 % for yeast samples from warm cropping. The data of trial 1 and 2 in Brewery A as well as the data from Quain et al. [3] only contain the first crop of the warm cropping procedure. Powell et al. [2] followed for the warm cropping not just the first crop but as well the second crop after diacetyl reduction and cooling down of the fermenting vessel. If only the data from the first crops are considered the findings for the viability are in agreement with the present data and Quian et al. [3] (approx. 88 to 94 % viability).

In Brewery B the crop of four fermentation vessels were followed (trials 1 to 4, Fig. 3 to 6). For all four trials the physiological conditions (vitality and viability) of the yeasts were far lower than in Brewery A.

In trial 1 the ICP showed almost no variation throughout the yeast crop but the values are at low end of the measure range of the method indicating very low yeast vitality. That this yeast is in poor physiological condition is supported by the low viability (74.8 ± 1.6 %). In Brewery B a green beer centrifuge is used on the transfer from the fermentation vessel to the storage tank. As an interesting aspect for this trial 1 the yeast which was recovered form the centrifuges was as well analysed for its physiological condition. Even if the vitality of this yeast can not be considered good it was with a value of 5.84 significantly higher than the yeast from the cone. The viability although was with 75 % at exact the same level of the cropped yeast from the cone. This indicates that the drop in vitality was due to the unfavourable conditions in the cone. It is important to notice that even if viability and vitality are connected the viability is not a sufficient parameter to indicate the overall physiological condition of a yeast culture.

In trial 2 it was possible to take a sample of the pitching yeast prior the fermentation as well as to follow the yeast crop at the end of fermentation. It turned out that the vitality of the pitching yeast was slightly above the vitality of the different samples from the crop and the vitality decreased slightly in the course of the yeast collection. It would be expected that the yeast vitality would increase during fermentation since new cells are formed in the growth phase of the fermentation. Apparently the vitality decreased again while the yeast stayed in the cone.

It is somewhat unexpected that the samples from the bottom of the cone (start of the crop) showed the highest vitality values since this yeast settled to the bottom first and therefore was exposed to all kinds of storage stress longer than yeast which settle down later in fermentation. This result is in contrast to the findings of Powell et al. [2] who found at least lower amounts of glycogen in the yeast from the lower parts. This can be explained by the additional stress the yeast is exposed to. In trial 3 and 4 the first yeast always showed relative high vitality compared to the later samples and especially in trial 3 the first sample showed a significant higher vitality value than all other samples of this trial (Fig. 5). A possible explanation for this is that the cells are basically activated by the stress and that they are in that moment in better physiological conditions but since they use up their glycogen reserves they are likely to die if these conditions persist. But this phenomenon should be investigated more in detail. That the higher vitality value correlates as well with a better fermentation performance can be seen in the fermentation profile of the following tall tube fermentations of trial 3 which will be discussed later.

The viability of the trials in Brewery B showed more variations than in Brewery A but the values are diverge around a certain level and they are in correspondence to the vitality values (trial 1: 74.8 ± 1.6 %; trial 2: 84.9 ± 2.8 %; trial 3: 85.8 ± 1.4 %; trial 4: 91.1 ± 0.9 %). And even if the viability in the studies of Quain et al. [3] and Powell et al. [2] showed more variations throughout the cone the differences are minor and therefore can be considered as correspondent.

3.2 Analysis of Barm Beer Surrounding the Yeast Cells

The cropped yeast slurries contain large amounts of beer. Since the environmental conditions have a major effect on the physiological conditions of yeast certain characteristics of the barm beer can indicate the level of stress the yeast is exposed to. Therefore the barm beer of the trial 2 (Brewery A) and trails 3 and 4 (Brewery B) were analyzed for the apparent extract, alcohol content (abv), pH and Free Amino Nitrogen (FAN) content. The alcohol content as a direct cause of stress as well as the apparent extract (or specific gravity) and the pH have been used in similar studies to characterize the environmental conditions. The FAN value has not been used for this purpose yet. However, but it has been found that especially this value increases in recoverable yeast beer with adverse storage condition due to excretion of amino acids and autolysis of yeast [30].

The most obvious difference between the results of Brewery A (Fig. 7) and the results for Brewery B (Fig. 8 + 9) was the last sample of each trial. For brewery A all parameters hardly change over all samples and for brewery B the last sample of each trial showed basically regular beer characteristics rather than the characteristics of recoverable yeast beer. These two samples contained substantially smaller amounts of solids and therefore the sample mainly constituted of beer (data not shown) which explains these results. Basically this means in brewery A the cropping process was stopped earlier than in brewery B to minimize beer losses.

In both breweries the results for the different analysis are quite constant over the course of cropping (apart from last sample from each crop in brewery B) and the results of both breweries are in general comparable. The abv ranges for almost all samples between 7 and 8 % which clearly shows that the yeast is exposed to severe ethanol stress in the cone of a fermentation vessel. Therefore the resident time should be kept as short as possible as suggested by numerous Authors [2, 3, 20, 31]. In comparison to the work of Quain et al. [3] and Powel et al. [2] the abv values are about 1 % lower. This can be explained by the fact that in both studies high gravity fermentations were followed (15 °P) while both breweries in the present study used normal gravity fermentation (12 °P and 11 °P respectively).

The apparent extract values for all samples (Brewery A and B) are below but close to 2 % with slightly lower levels for trial 4 in brewery B. The pH-Values are around 6 with again the exception of trial 4 (brewery B) with values around pH 5. Quain et al. [3] and Powel et al. [2] found usually values between 4.5 and 5.0 with exception of the first crop of the two step warm cropping regime. A possible explanation for these differences is that the pH in the barm beer is mainly influenced by the yeast strain. Another influencing factor for the high pH could be the wort. High gravity worts with adjuncts show lower pH than all malt worts. The present results of the two completely distinct breweries are quite comparable and both use the same yeast strain. In the works of

Quain et al. [3] and Powell et al. [2] the same yeast strain (BB11) has been used and it is likely that both works have been executed in the same brewery.

Greater differences have been found for the FAN values of the different breweries and the different trials. The lowest values where found in brewery A (Fig. 7) with just under 200 mg/l for all samples. In trial 4 (brewery B; Fig. 9) the contents range from 200 to 230 mg/l and in trial 3 (brewery B; Fig. 8) values of 270 to 340 mg/l were found. If these values are compared to the respective vitality levels (Fig. 2, Fig. 6 and Fig. 5 respectively) it becomes clear that the higher the vitality the lower the FAN-Values. This does not mean that the FAN measurement can be directly used as a vitality measurement. But in thick yeast slurries FAN accumulates due to excretion of amino acids by yeast and autolysis of cells which can be used under certain circumstances (same yeast strain, same yeast concentration; e.g. in one brewery) as an indicator of the physiological conditions.

In the works of Quain et al. [3] and Powell et al. [2] for the conventional crop (yeast crop after diacetyl is below the threshold. and cooling down of fermentation tank) barm beer characteristics of the second half of the crop change notably (abv dropped by 1 %; pH dropped approx. half a unit; specific gravity increased). In brewery B of the present study the yeast crop followed as well the conventional cropping regime but similar tendencies for the abv, pH and specific gravity were not found. The reason for this discrepancy is not known but the different yeast strains might show different characteristics in the way they accumulate in the cone. Apparently strain BB11 which was used by Quain et al. [3] and Powell et al. [2] does not accumulate as compact in the upper regions of the cone and therefore more residual beer is present which causes the differences in the analysis.

3.3 Cell Age and Fermentation Performance of the Different Samples

Deans et al. [1] as well as Powell et al. [2] investigated the cell age distribution throughout the cones of industrial fermentations. Both studies found great variations within the different layers of the cone. But their results differed considerably. Deans et al. [1] found an age gradient with the oldest cells accumulated at the bottom of the cone and an increasing percentage of younger cells towards the top layers. Powell et al. (2) found the oldest cells accumulating in the middle of the cone.

For the present study only the trial 3 and 4 (brewery B) were analysed for their cell age distribution. The results are shown in Figure 10 and 11.

The cells were grouped in classes according to the number of bud scars. Cells with no bud scar as well as the group with one and two bud scars are clearly dominant in these trials. The proportion of the group without bud scars is increasing to the later samples (toward the top layers of the cone) in both trials. Only the last sample which has been taken did not fit into this pattern and it showed basically the same distribution as the starting sample. The analysis of the barm beer already indicated that a high proportion of beer was mixed with the yeast in the last sample of each of the two trials and it can therefore be assumed that the last sample did not represent the top layer of the cone.

The results support the findings of Deans et al. [1] which suggested that younger cells sediment slower to the cone than older cells. Yeast flocculation supposedly has the strongest influence on the sedimentation rate. But the general cell size as well as other factors have any influence on the sedimentation of cells. Therefore it is likely that different age distributions can be found in cones of industrial fermentations (e.g. results of Powell et al. [2]).

Deans et al. [1] used the yeast samples collected at different times of the yeast crop to investigate if the different cell age distribution as well influences the fermentation performance. They found that the yeast collected at the mid point of the yeast crop representing a mixture of old and young cells the best fermentation performance and that there was hardly any difference between the bottom layers and the top layers. In order to investigate the same aspect the yeast samples of trial 3 and 4 (brewery B) were used to pitch EBC tall tubes (in duplicate) with commercially produced wort at a rate of 12 Mio cells/ml.

The fermentation profiles of the two trials are shown in Figure 12 and 13.

In general the fermentation rates of the different samples did not differ very much. Only the start sample in trial 3 (Fig. 12) showed a notably faster fermentation compared to the other samples. Quain et al [3] presented evidence that actually the older and larger cells show higher fermentation rates than younger cells which is in contrast to the findings of Deans et al. [1]. This could be used to explain why the start sample in trial 3 performed better than the other samples since in this sample the oldest cells were found. However, the sample after 26 hl showed the same cell age distribution than the start sample (Fig. 10) although not the same good fermentation performance. Therefore other factors must be responsible for this effect. Interestingly this start sample of the yeast crop representing the bottom layer of the cone showed a significant higher vitality value than the other samples from the same trial (Fig. 5) and this can explain the notably better fermentation performance.

The differences between the fermentation profiles of the trial 4 (Fig. 13) are minor with a tendency of faster fermentations for the samples taken at the end of the crop. The cell ages again can not explain these results since the start sample and the last sample (after 26 hl) had the same age distributions but performed quite differently in the fermentation. The differences in vitality as well can not explain the fermentation performance but ICP values ranging form 5.95 to 6.15 anyway do not represent major differences.

Deans et al. [1] investigated the resulting beer of the tall tube fermentation on their content of several volatile components. Only a few of these volatiles showed differences. Other volatiles compounds were investigated in the present study, however hardly any differences were found. The largest variation was found for the ester Ethylacetat with 28.1mg/l (± 5.1 mg/l; Least Significant Difference (LSD); Confidence Level 0.95) for trial 3 (Fig. 14) and 35.0 ± 4.0 mg/l for trial 4 (Fig. 15).

The differences in the absolute values between the two trials are due to different worts and therefore these differences should not be discussed. In both trials the start sample of the crop always showed notably lower values in Ethyl-acetate than the other samples even if the start samples showed the same cell age distribution as the last sample of each trial. The reasons for these results are not known but it is possible that this phenomenon is related to stress to which the yeast is exposed especially in the lower region of the cone.

Isoamyl-acetate showed a similar pattern with the lowest values for the start samples even if the variations have been not as great (trial 3 = 2.7 ± 0.4 mg/l; trial 4 = 4.0 ± 0.3 mg/l). All other volatile components showed hardly any variations like 2-Methyl-Propanol (trial 3 = 10.4 ± 0.4 mg/l; trial 4 = 14.3 ± 0.6 mg/l) or 3-Methyl-Butanol (trial 3 = 44.6 ± 1.5 mg/l; trial 4 = 49.5 ± 0.6 mg/l).

4 Conclusions

In this study quality parameters of yeast samples collected during vessel cropping in two breweries have been investigated. The study included crops of yeast in very good, average and very poor physiological condition. Independent of this overall physiological condition of the yeast vitality and viability did not vary considerably throughout the individual crops representing the different layers of the cone. Therefore it is not possible to select specifically good quality yeast by cropping certain layers of the yeast in the cone. The characteristics of the recoverable beer from the yeast crop as well did not show great variations within the cone. But high levels of ethanol and increasing excretion of FAN with decreasing vitality levels indicate that environmental conditions in the cone cause severe stress to yeast and therefore an early yeast crop can help to maintain healthy yeast.

In respect to the cell age it was found that in both breweries (using the same yeast strain) older cells accumulated in the bottom part of the cone with an increase of younger cells towards the top layers of the cone. Successive cropping and repitching of certain layers could lead to undesired selection of yeast cells with certain characteristics. However, influences of the cell age on the yeast performance as found in other studies could not be confirmed. Several results obtained in this study (cell age gradient, recoverable yeast beer characteristics etc.) are not in accordance with other studies which seem largely due to the individual characteristic of the different yeast strains involved.

Although only minor variations within a single yeast crop have been found regarding yeast vitality and viability the differences in-between crops have been huge even within one brewery showing the importance of a good yeast management and the need for more investigation in this field.

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Appendix

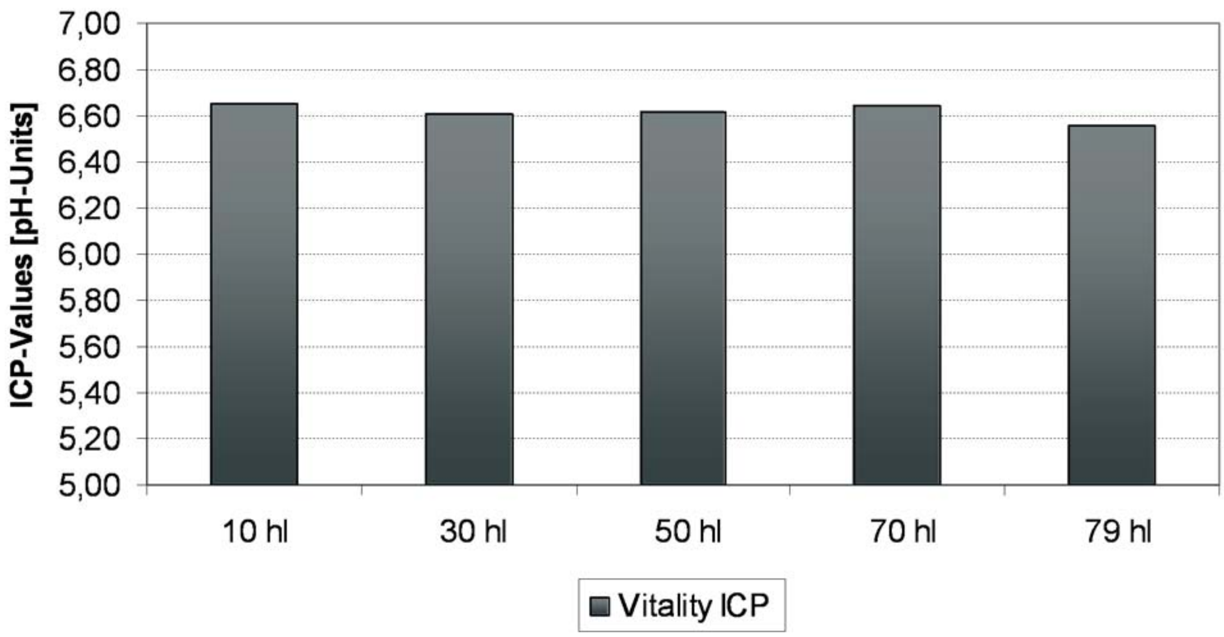

Figure 1 Yeast vitality of samples from yeast crop in Brewery A (trial 1) taken at the indicated volumes of the total crop

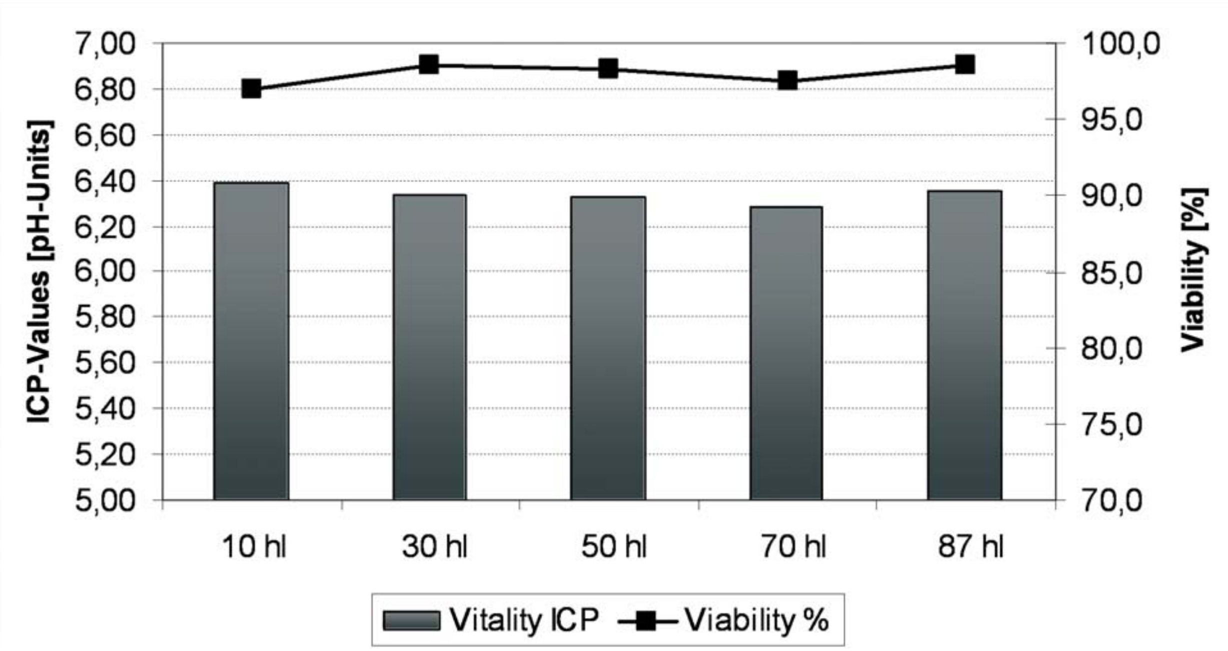

Figure 2 Yeast vitality and viability of samples from yeast crop in Brewery A (trial 2) taken at the indicated volumes of the total crop

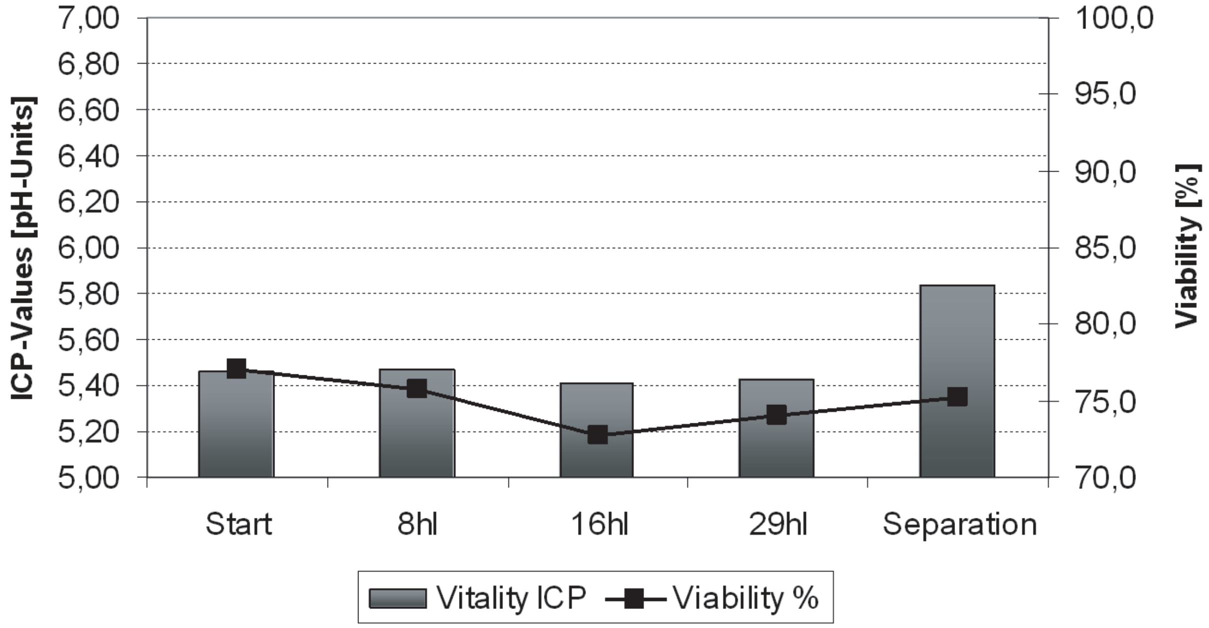

Figure 3 **Yeast vitality and viability of samples from yeast crop in Brewery B (trial 1) taken at the indicated volumes of the total crop including a yeast sample (Separation) from the green beer centrifuge**

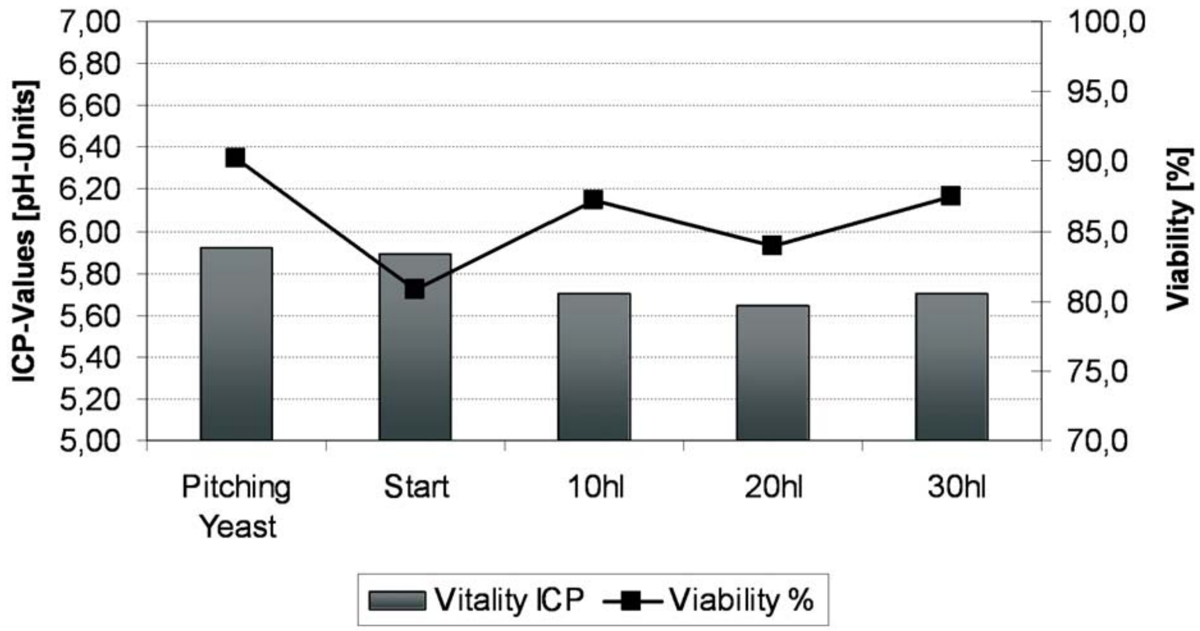

Figure 4 **Yeast vitality and viability of samples from yeast crop in Brewery B (trial 2) taken at the indicated volumes of the total crop including the vitality and viability of the yeast the tank was pitched with**

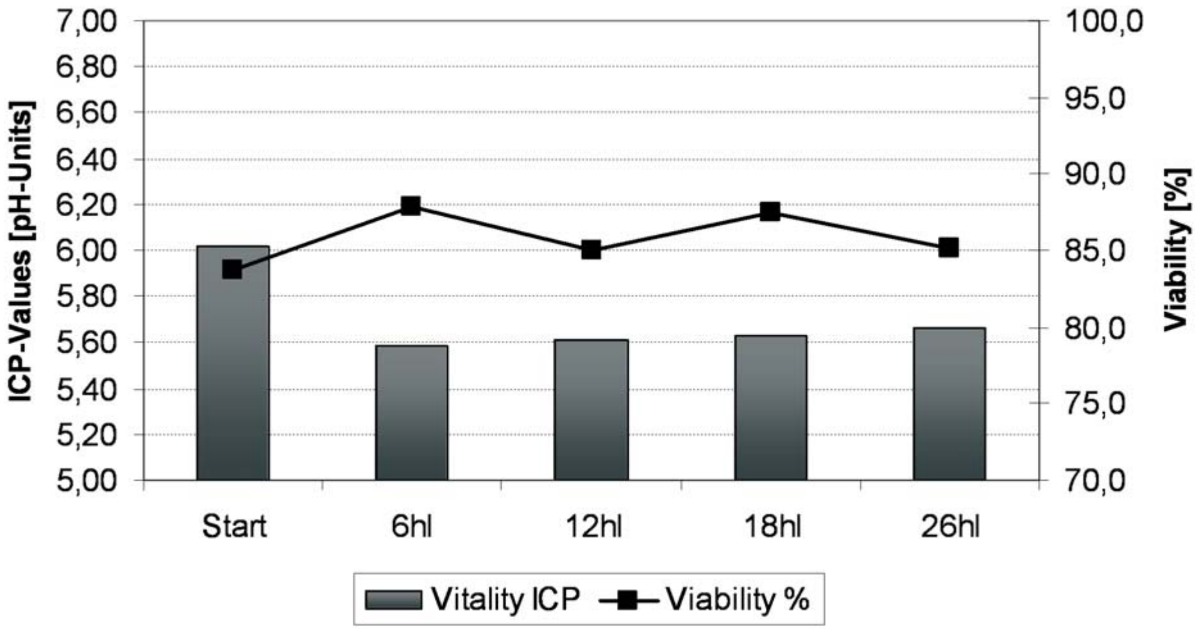

Figure 5 **Yeast vitality and viability of samples from yeast crop in Brewery B (trial 3) taken at the indicated volumes of the total crop**

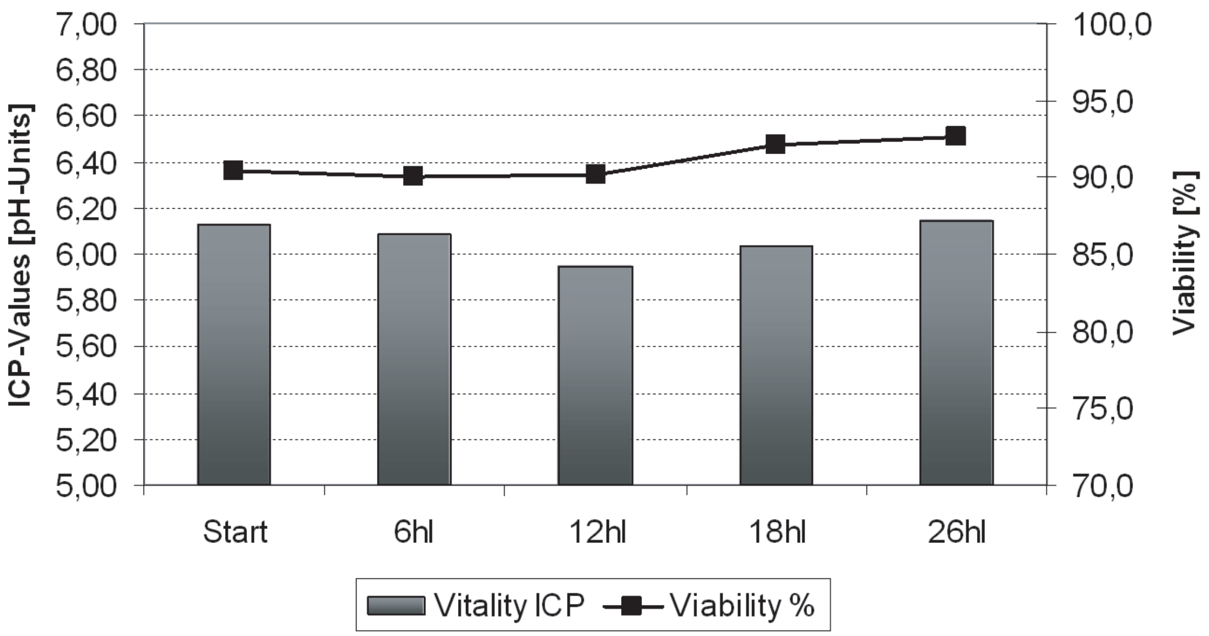

Figure 6 **Yeast vitality and viability of samples from yeast crop in Brewery B (trial 4) taken at the indicated volumes of the total crop**

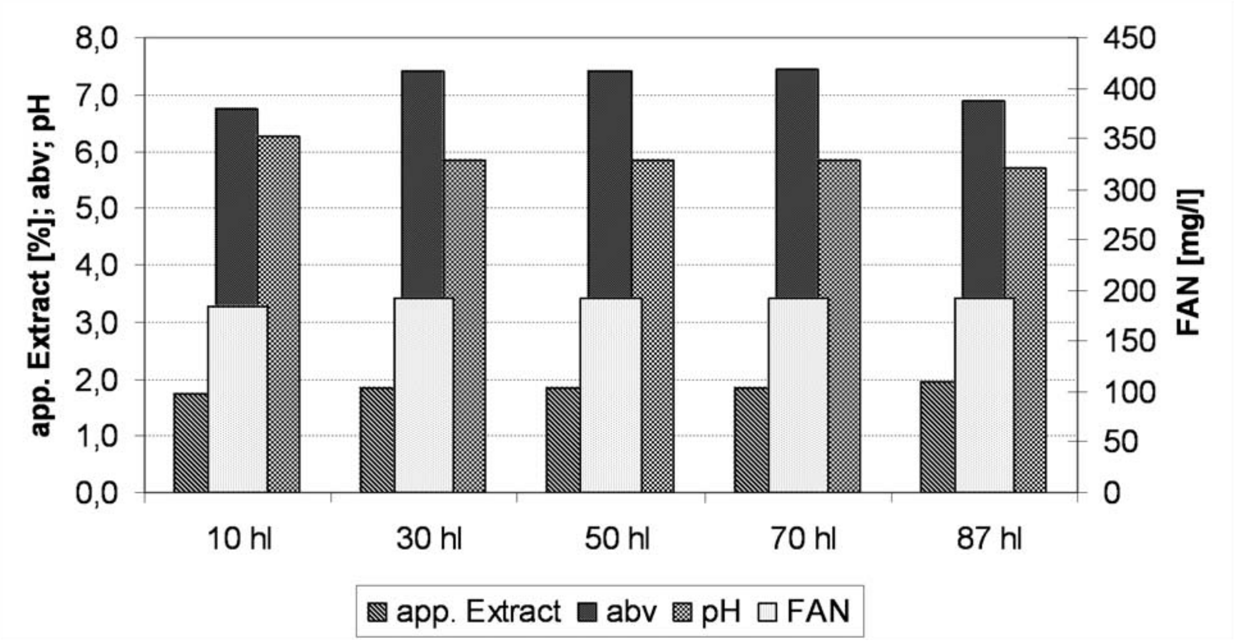

Figure 7 **Analysis of the barm beer from the yeast samples taken at the indicated volumes of the total crop in Brewery A (trial 2)**

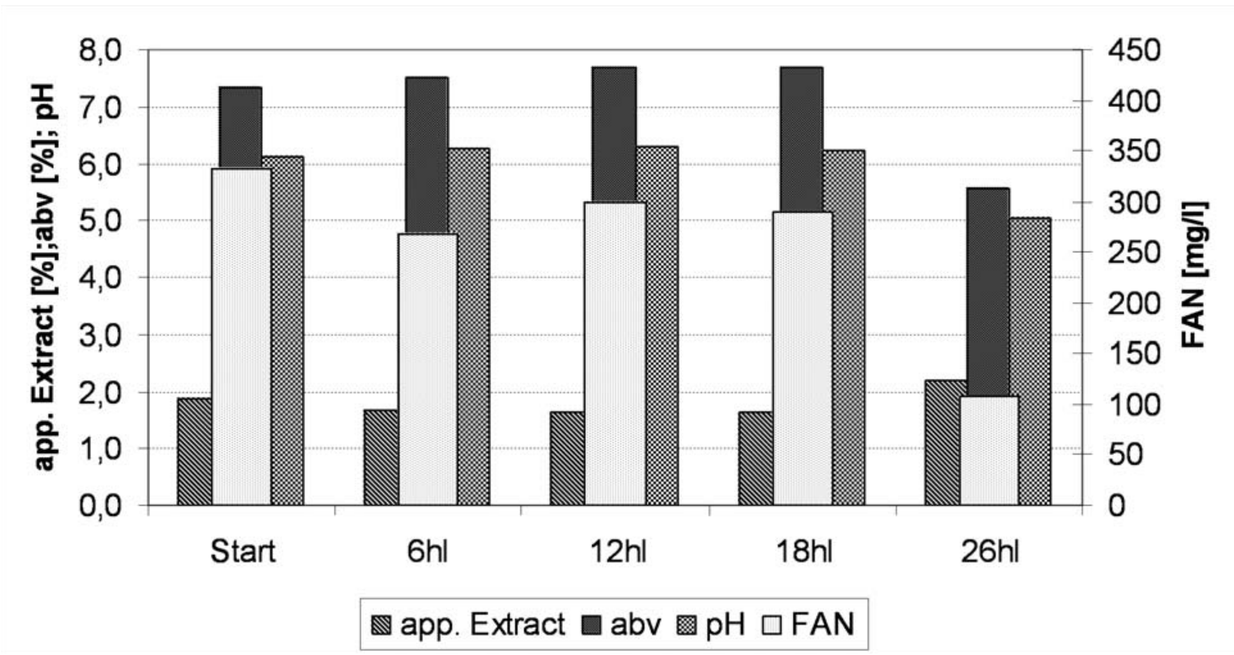

Figure 8 **Analysis of the barm beer from the yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 3)**

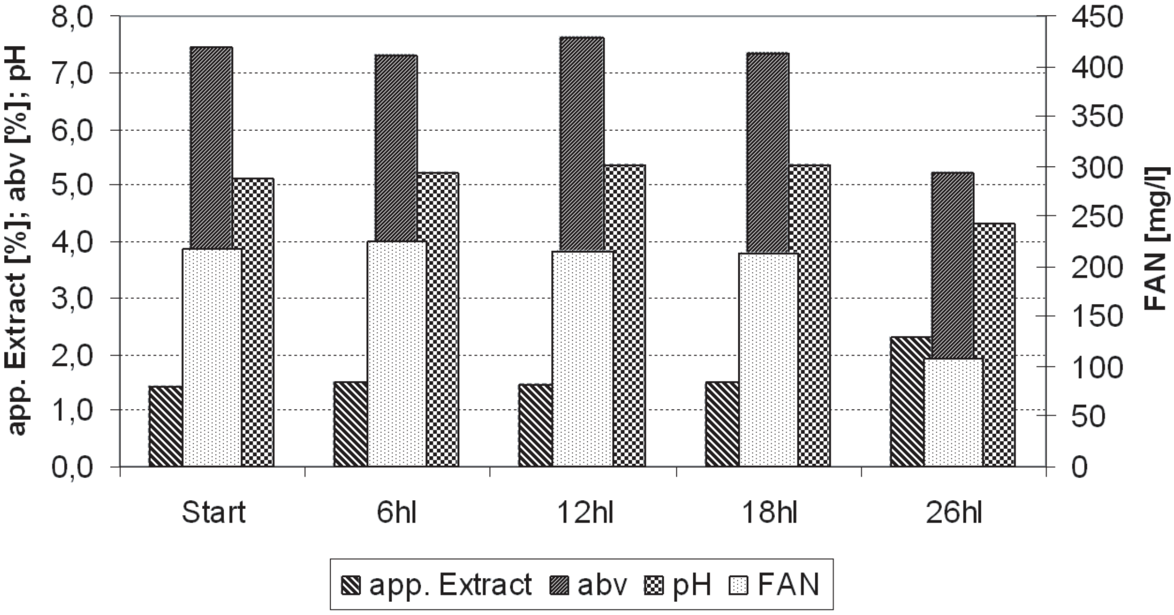

Figure 9 **Analysis of the barm beer from the yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 4)**

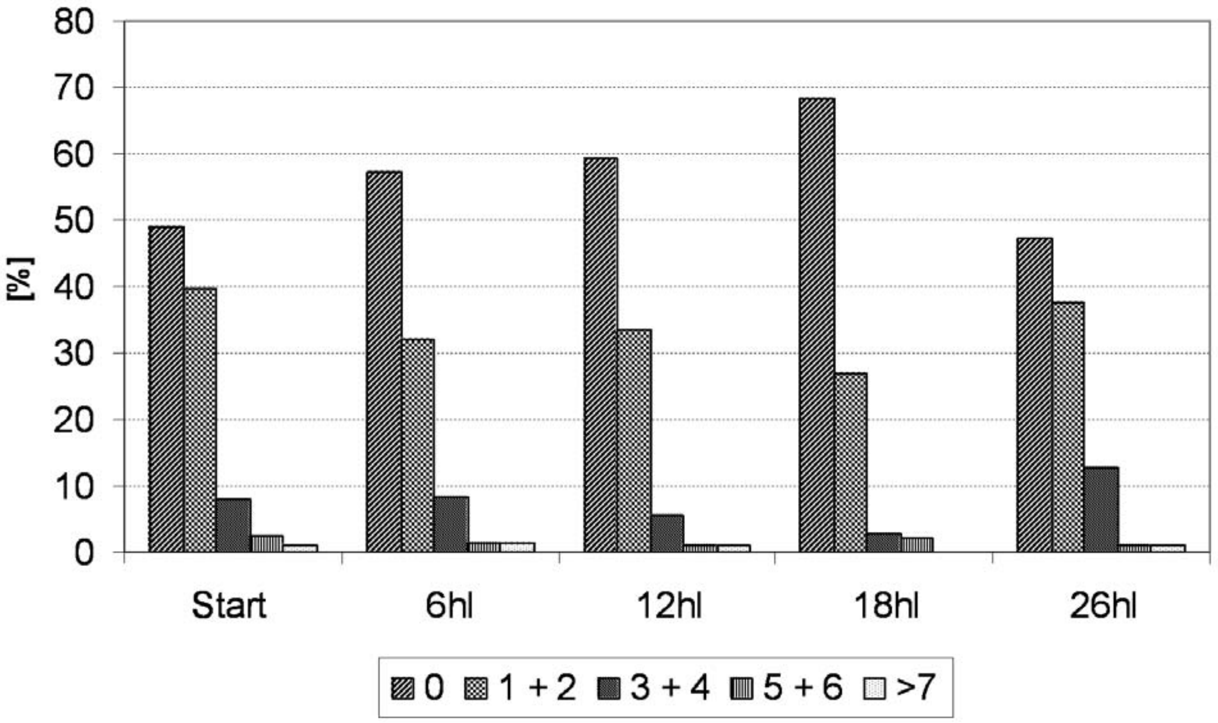

Figure 10 **Analysis of the cell age distribution of the yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 3)**

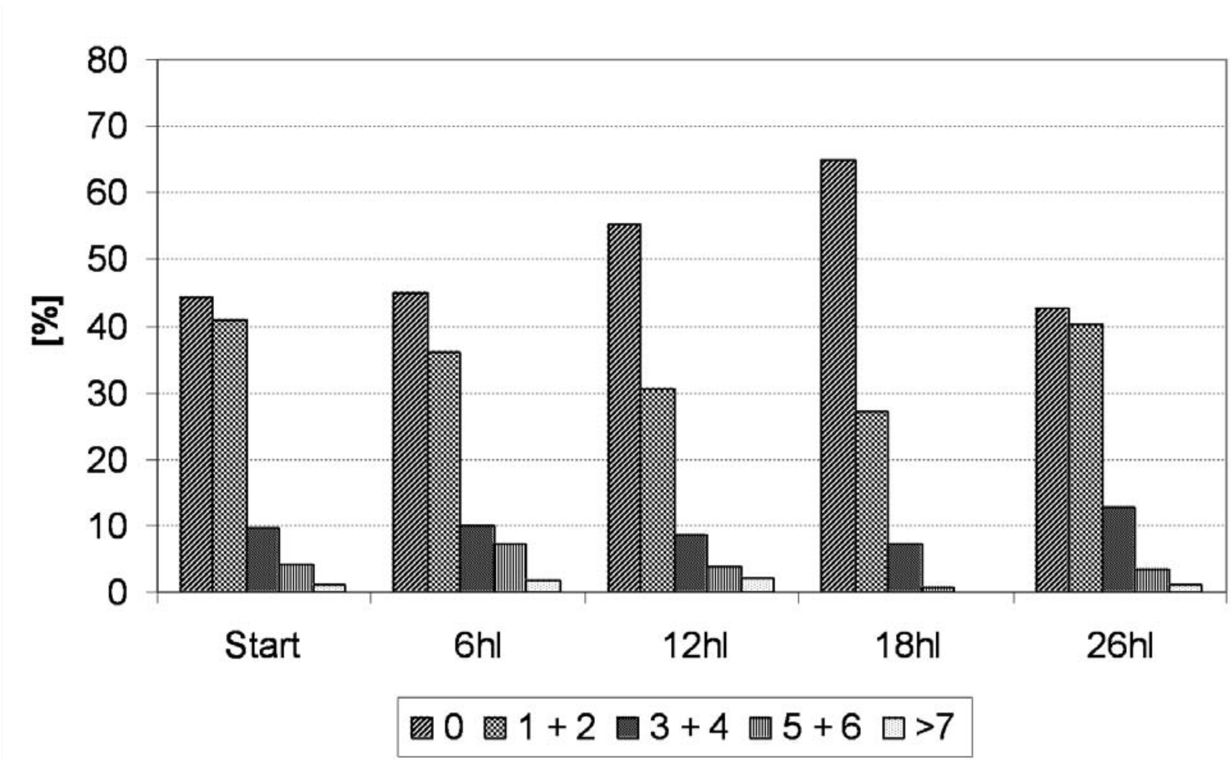

Figure 11 Analysis of the cell age distribution of the yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 4)

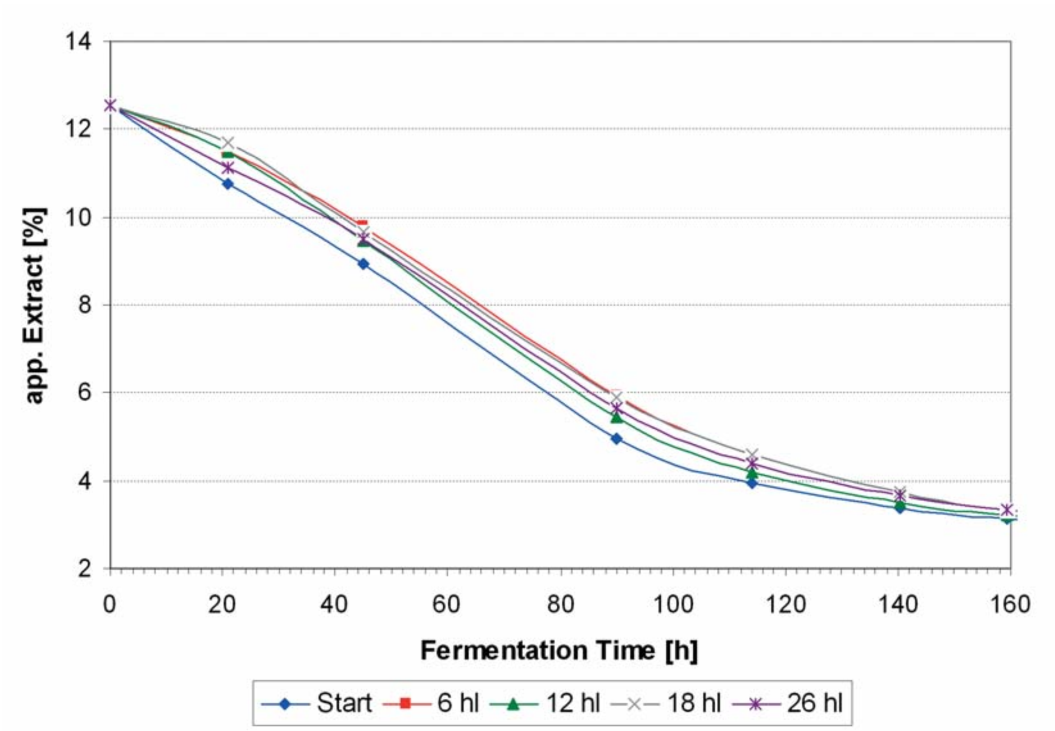

Figure 12 Fermentation performance of tall tubes pitched with the yeast of the cropped yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 3)

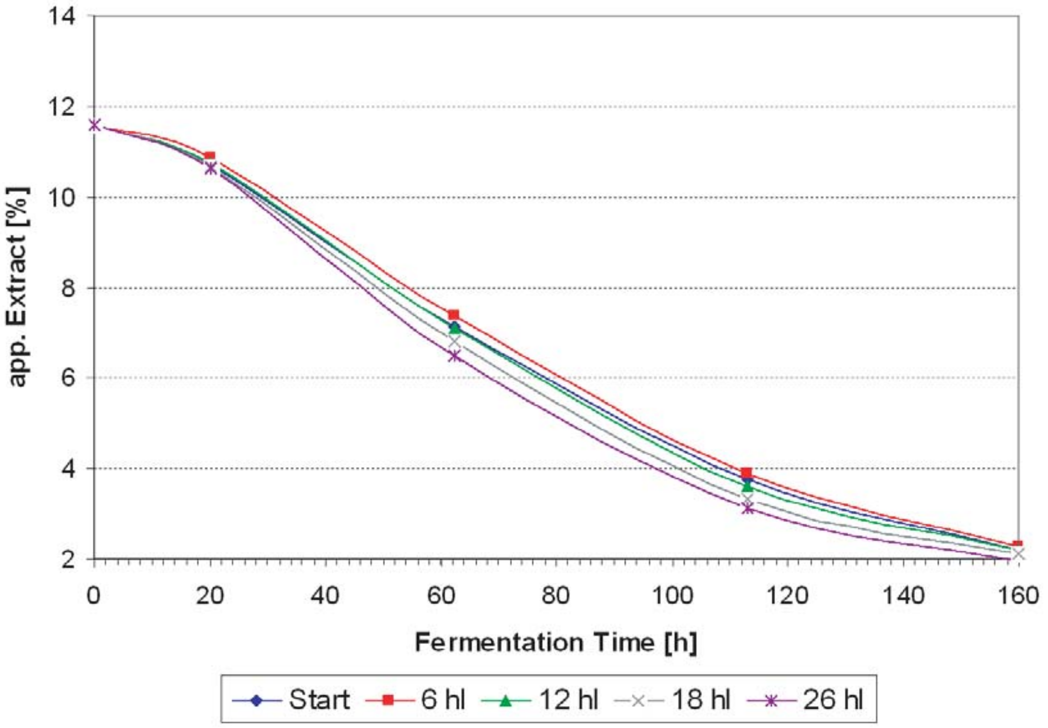

Figure 13 **Fermentation performance of tall tubes pitched with the yeast of the cropped yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 4)**

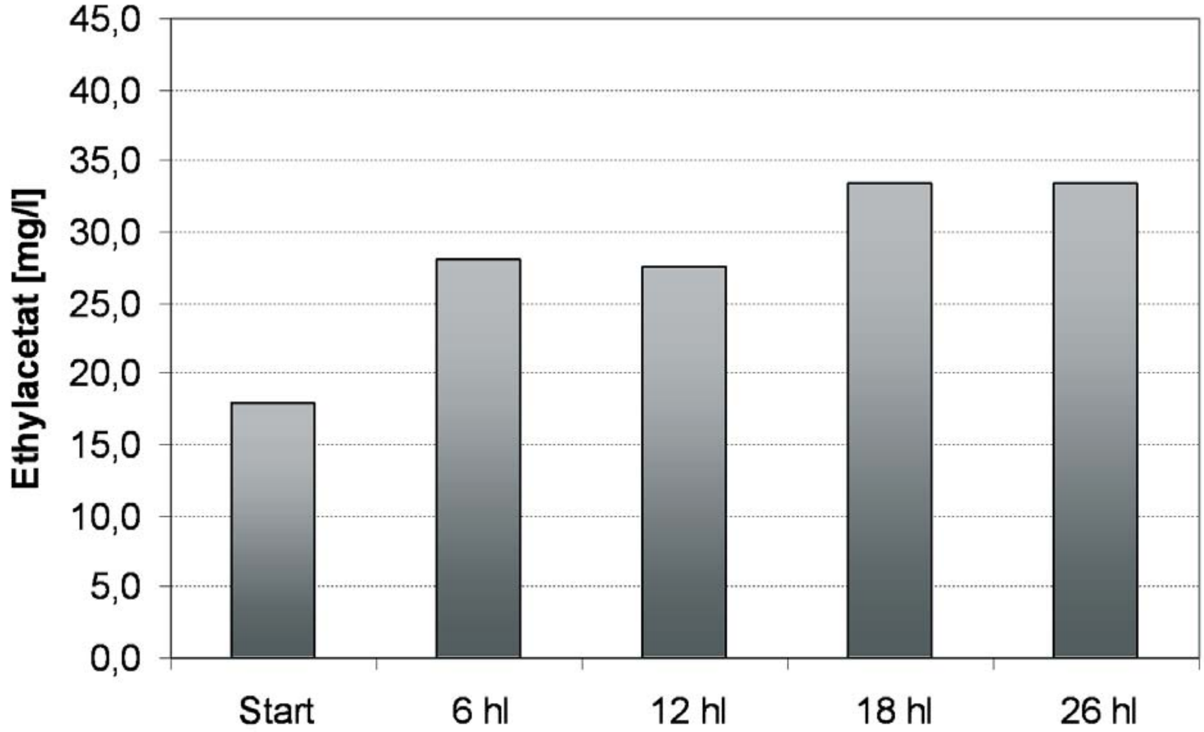

Figure 14 **Ethyl acetate concentration of the beer resulted of the tall tube fermentations with the different yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 3)**

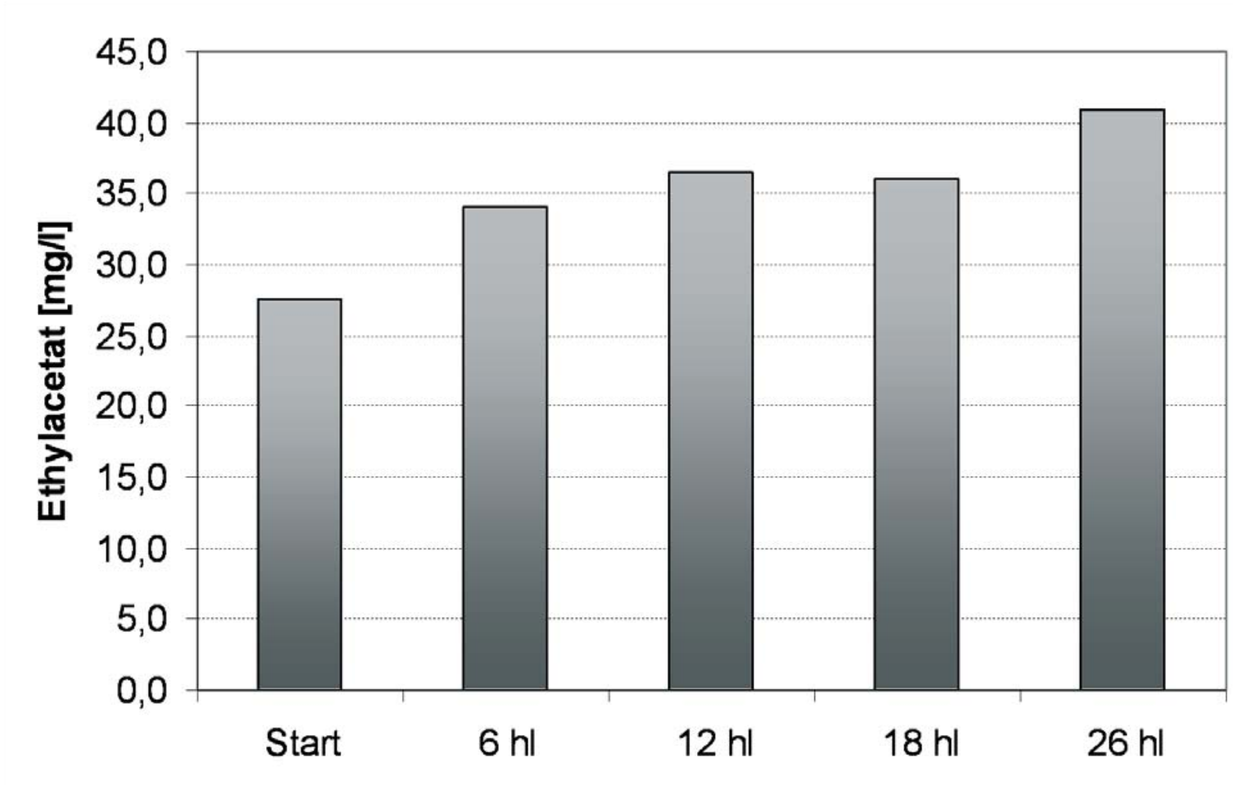

Figure 15 Ethyl acetate concentration of the beer resulted of the tall tube fermentations with the different yeast samples taken at the indicated volumes of the total crop in Brewery B (trial 4)