K. Müller-Auffermann, A. Caldera, F. Jacob and M. Hutzler

Characterization of Different Bottom Fermenting *Saccharomyces pastorianus* Brewing Yeast Strains

Brewing yeast plays a pivotal role in determining the flavor and quality of beer. Therefore, it is crucial that reliable and practical information be obtained regarding the characteristics of individual strains. This paper presents a comparison of six commercially available bottom fermenting yeast strains. TUM 34/70 served as the standard against which these yeast strains were compared, since it is the most widely used bottom fermenting yeast strain in the world and is also the most comprehensively described scientifically. A fermentation plant consisting of 27 fermentors was developed and tested with this goal in mind. This plant was designed to allow fermentation to be controlled according to a set of variables which included temperature, aeration, cell count and pressure. For the purpose of carrying out fermentation trials, these fermentors provide conditions comparable to those found in large industrial tanks.
In three separate sets of trials, the following attributes were measured at regular intervals during fermentation and after lagering: the drop in specific gravity and in pH, CO_2 production, FAN reduction, residual maltotriose content, total yeast cell count, the number of cells in suspension, the surface charge of the cells, turbidity, sedimentation and the foam stability of the unfiltered beer, as well as the formation and degradation of vicinal diketones, fusel alcohols, esters, aldehydes and SO_2. Finally, a proper organoleptic description comparing the unfiltered products was performed after lagering.
In doing so, the strains were tested under conditions as close as possible to those present in actual brewing operations and thereby exhibited, in part, significant differences in their behavior. Moreover, new insights have been gained which had yet to be fully described scientifically. These include the change in the surface charge on the yeast cells and the absorption rate of certain amino acids during fermentation, which occur independent of a specific yeast strain.

Descriptors: yeast, brewing yeasts, *Saccharomyces pastorianus*, bottom fermenting yeast strains, lager, yeast characteristics, characteterization, TUM 34/70, TUM 34/78, TUM 193, TUM 194, TUM 66/70, TUM 44, TUM 69

1 Introduction

The quality of the raw materials and the wort, as well as effective management of the processes used in beer production, including those downstream processes employed in fermentation and on through to bottling, are of the utmost importance for producing sensorially flawless beer of the highest quality possible. Fermentation, in particular, exerts a major influence on the flavor profile, the appearance and the chemical and physical stability of the finished beer, due to the many biochemical reactions that take place during this process [1]. It is well known that, aside from the composition of the substrate, fermentation performance can be influenced through technical and technological means. Such factors include head pressure, convection, temperature, aeration rate and the yeast strain selected. In this case, the rheological behavior of the yeast must also be taken into consideration, in order to respond with technical measures when yeast, for example, undergo flocculation and sedimentation.

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On the whole, it is therefore imperative that commercially available brewing yeast strains be precisely characterized and described with respect to their properties according to defined and standardized parameters. As early as 1930, [2] a methodology for distinguishing the fermentative power of different yeast strains was developed by tracking the drop in specific gravity by means of pycnometry. Concurrently, an apparatus consisting of several pear-shaped vessels was used to quantify the carbon dioxide formed during fermentation, in order to establish a strain-specific Halbgärzeit, as it is referred to in German [3]. This term refers to the time required for half of the volume of CO_2 to be produced which is theoretically possible at a given temperature and under certain conditions. Another line of inquiry was explored with the intention of gaining meaningful insight into high and low attenuating yeast strains [4]. A method more relevant for industrial production practices was developed to determine various key parameters in the process which could be measured even while fermentation was in progress. Subsequently, a cylindrical fermentation vessel featuring a central

conical section was employed from which samples could be taken for further analysis [2]. A method based on monitoring the increase in head pressure during fermentation was developed for the selection and detection of brewing yeast under controlled conditions. This allowed the yeast, which tends to remain in suspension, to be differentiated from flocculent yeast [5]. These methods, however, proved to be unsatisfactory [2]. Precisely how different factors in the fermentation process are interconnected could not be achieved using these methods. Furthermore, they were often not suitable for practical applications or for statistical analysis, since only one fermentation apparatus was generally used per trial and per yeast strain. Additionally, the resultant data on the yeast strains thusly characterized were often of limited relevance, since the results were typically only of interest to specific breweries, given that the yeast subject to analysis was often not available for purchase commercially.

In 1987 and 1991 bottom and top fermententing brewing yeast strain were characterized and compared in pilot scale fermentations with a focus on practical applications of the yeast strains [6–8]. Lateron only few publications described brewing yeast strains from a practical and technological point of view so that data can be used to choose an appropriate yeast strain or to modify the process to optimize yeast strain performance [9–11]. The goal of this paper is therefore to examine and describe to the greatest extent possible the commercially available bottom fermenting yeast strains TUM 34/78, TUM 193, TUM 194, TUM 66/70, TUM 44 and TUM 69 from the Yeast Center at the Weihenstephan Research Center for Brewing and Food Quality with regard to their fermentation properties under practical, standardized fermentation conditions in the laboratory. TUM strain 34/70 served as the reference strain for evaluating the fermentation properties of these yeasts, as it has been very accurately described scientifically and has also been utilized more than any other strain for the production of lager beer (helles, pilsner) [12].

2 Review

Fermentation activity as well as the sensory attributes of the beer can be characterized by regularly taking measurements and by tracking various data points during production. These play a crucial role in ensuring the quality of the final product and in establishing reliable and consistent production processes.

2.1 The drop in specific gravity, degree of attenuation and sugar utilization

Sugar assimilation is the most important aspect of fermentation. The two primary products of this process are ethanol and CO_2. During fermentation, the yeast consumes fermentable sugars, the concentration of which can most readily be determined by regularly measuring the density of the liquid and the alcohol concentration. The degree of attenuation is not a quantifiable unit. It can, however, be calculated as a percentage of the drop in specific gravity over the original gravity of the pitching wort.

Not all sugars present in the wort are fermentable, and not all of the fermentable sugars are consumed uniformly or at the same time. While glucose (approx. 12 g/L in a 12 % wort), fructose (approx.. 2,5 g/L) and sucrose (approx. 4 g/L) are immediately metabolized by brewerythe yeasts, they absorb and utilize maltose (approx. 74 g/L) and maltotriose (approx. 17 g/L) much more slowly [13]. To what degree the maltotriose in the wort is taken up and metabolized by the yeast depends on the condition of the cells and yeast management practices [14]. However, *Dietvorst* discovered that maltotriose transport and utilization are also specific to each yeast strain [15, 16].

2.2 The drop in pH over the course of fermentation

The hydrogen ion concentration, known as the pH value, has a major impact on many aspects of the entire brewing process and therefore on the quality of the finished beer. In the work done for his dissertation, *Piendl* was able to detect differences in flocculent yeast and the yeast remaining in suspension with respect to their capacity to produce acid and thus their ability to reduce the pH of the fermenting wort. His results show that flocculent yeasts do indeed bring about a reduction in pH more rapidly than the yeast remaining in suspension, but the final pH value was ultimately higher by comparison [2].

2.3 Amino acid uptake and utilization

The yeast must be supplied with amino acids in order to facilitate the construction of cellular proteins, especially during the log phase. In this phase of growth, the organisms remove the amino groups from the wort amino acids through transamination and utilize them to create their own amino acids from organic acids formed in the yeast cells [13]. The amino acid uptake is performed actively using permeases. Some of them are specific to individual amino acids while others are non-specific [14]. Thus far, a total of 16 different amino acid transport mechanisms are known [18]. The amino acids were classified by *Pierce* according to the order in which they are taken up from the wort. The amino acids in group A are absorbed quickly, and those in group B, more slowly yet continuously, while the amino acids in group C are taken up only after those in group A are consumed. Group D, however, according to Pierce consists exclusively of proline which he deemed not to be taken up by the cells at all. Nevertheless, recent studies have recommended that the classification of amino acids according to Pierce be revised, specifically that the amino acids isoleucine, leucine and methionine be removed from group B and placed in group A, while certain amino acids in group C, namely tyrosine, tryptophan and phenylalanine, be reassigned to group B [19, 23].

2.4 Fermentation by-products (FBP)

The formation of fermentation by-products (FBP) is essential, as they are responsible in large part for the quality and sensory characteristics of the finished beer. For this reason, the following FBP were determined as part of the work presented in this paper:

- SO_2: a reducing agent and oxygen scavenger, thus this compound has a positive effect on flavor stability. The risk of oxidation increases when the concentration of SO_2 is too low (<2 ppm).

- **Diacetyl/vicinal diketones (VDK):** their character is described as unclean, possessing a sweet flavor of butter or butterscotch. These substances have extremely low sensory thresholds: total diacetyl: <0.15–0.20 ppm and pentane-2,3-dione: <0.5–0.6 ppm.
- **Acetaldehyde (aldehydes):** at high concentrations, this substance produces a flavor reminiscent of green apples. Its flavor threshold is generally 25 mg/l; however, in beer with a less pronounced aroma, it can be as low as 12 mg/l [13].
- **Higher alcohols:** these substances are flavor-active and possess a flavor threshold of approximately 100 mg/l. At high concentrations, an unpleasant bitterness results, and the palatability of the beverage is compromised.
- **Esters:** these are considered the most important constituents of aroma in beer. Esters represent a diverse group of aroma-active compounds. At high concentrations, they generate a sharp, pungent odor and an unpleasant, bitter flavor, perceived along with a fruity character.

2.5 Cell count

Yeast cells in suspension are crucial for achieving a desirable degree of attenuation and for eliminating the aroma substances found in green beer towards the end of primary fermentation. However, if too many yeast cells are in suspension following lagering, this can have an adverse effect on the filterability of the beer [13]. To this end, knowledge of the flocculation and sedimentation properties of a particular strain plays a fundamental role in determining whether it is suitable for use in a brewing facility. *Hoffmann* was able to differentiate yeast according to their sedimentation behavior by assessing the volume of CO_2 they produce while fermenting at 25 °C. According to the data he collected, the yeast in suspension (non-flocculent) produced more CO_2 than the flocculent yeast [5]. Piendl, on the other hand, distinguished the sedimentation behavior of the yeast simply by recovering the volume of yeast collected in the lowest part of the fermentor [2]. In order to find out how many yeast cells were in suspension, he took samples from 3 cm below the surface and determined the concentration of cells using a Thoma counting chamber.

2.6 Surface charge

In potentiometric titration, a suspension with a complementary charge is titrated into another until the charge is neutral. According to the manufacturer, this type of titration is suitable for particles between 0.3 nm and 300 mm. In the brewing industry, potentiometric titration has been tested as an alternative for determining the chemical and physical stability of beers [20] and for determining yeast vitality [21]. As part of the work presented in this paper, the surface charge of the cells in suspension during fermentation was evaluated.

3 Materials and methods

3.1 Materials

The yeast strains tested in these trials were taken from agar slants and each transferred to two 100 ml flasks containing sterile wort. The flasks containing the inoculated wort were shaken (60 rpm) for three days at ambient temperature (20 °C) and pressure. Subsequently, they were each transferred into 2.5 liters of sterile wort and propagated while shaking for three more days at ambient temperature (20 °C) and pressure. After allowing six hours for sedimentation under identical conditions, the supernatant, which had reached the limit of attenuation, was decanted, and the yeast sediment in each container was transferred into 5 liters of sterile wort and further propagated. Once propagation was complete, the flasks were cooled to pitching temperature (15 °C), in order to allow the yeast to acclimate and sediment out. In three separate series of trials, TUM 34/78, TUM 193, TUM 194, TUM 66/70, TUM 44 and TUM 69 were compared with TUM 34/70 – always two of the strains named above together with the reference strain.

Standard-strength pale wort ("Original", a helles from the Staatsbrauerei Weihenstephan) was used as a substrate for fermentation and was brought to 15 °C prior to pitching the yeast. The wort possessed a mean original gravity of 11.8 °P and a FAN content of 11.7 mg/100 ml.

The equipment used for the trials consisted of 27 stainless steel vessels (diameter: 10 cm, height: 33 cm, volumetric capacity: 2 liters, headspace: 35 %; lids are clamped down) (see Figure 1 at left). The tanks are positioned in a cooling chamber and connected by coupling devices to a bung apparatus. The large number of vessels allows three vessels per yeast strain to be removed daily for sampling without this affecting the fermentation processes in the other vessels (see Figure 1 at right). Three yeast strains were tested simultaneously in each trial: the reference strain (TUM 34/70) and two more bottom fermenting yeast strains. The wort was mixed until homogeneous and inoculated with 30×10^6 cells/ml at a pitching temperature of 15 °C. During fermentation, a head pressure of 0.5 bar was set to simulate a liquid height of 10 m (median hydrostatic pressure). Fermentation

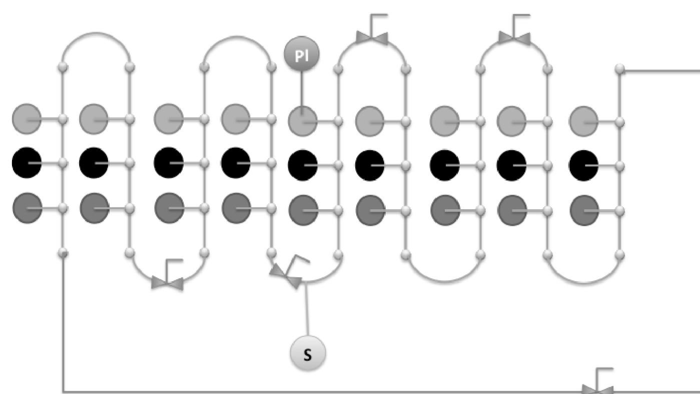

Fig. 1 The design of the pilot plant (S = bung device, PI = pressure indicator; each color signifies a row of fermentors containing the same yeast strain)

lasted four days followed by seven days for maturation at 4 °C under the aforementioned conditions.

3.2 Methods

Table 1 provides information regarding the point at which the samples were taken and according to which analysis methods they were evaluated.

Table 1 Analysis report for evaluating fermentation parameters

Parameter	Pitching yeast	Pitched wort	Day 1 of fermentation	Day 2 of fermentation	Day 3 of fermentation	Day 4 of fermentation	Lagering	Method
CO_2 production			X	X	X	X	X	See 4.2.1
Extract		X	X	X	X	X	X	MEBAK II 2.10.6.3
FAN		X	X	X	X	X	X	MEBAK III 3.3
pH		X	X	X	X	X	X	MEBAK II 2.14
Sugar spectrum		X				X	X	MEBAK III 3.2.1
Limit of attenuation			X	X	X	X	X	MEBAK II 2.4
Yeast cell count (total)	X	X	X	X	X	X	X	See 4.2.2
Cells in suspension			X	X	X	X	X	See 4.2.2
Surface charge	X		X	X	X	X	X	See 4.2.3 & [21]
Turbidity*			X	X	X	X	X	MEBAK II 2.15.1.2
VDK (total)			X	X	X	X	X	WBBM 2.21.5.1
VDK (free)							X	MEBAK III 1.2.1
FBP						X	X	WBBM 2.21.1
SO_2						X	X	WBBM 2.21.8.1
Sensory analysis							X	See 4.2.4

*measured parallel to the fermenters in stability bottles with the same pitched wort

3.2.1 Determining the amount of CO_2 produced during fermentation

The amount of CO_2 was determined gravimetrically by weighing the fermentors prior to opening them for removing samples or for further handling. The loss of CO_2 upon opening the vessels was calculated using the following equation:

$$CO_2\left(\frac{g}{100g\ extract}\right) = \frac{loss\ in\ weight\ (kg)}{full\ weight(kg).OG(\%-w/w)}.100 = \frac{initial\ weight(kg) - weight(kg)}{full\ weight(kg).OG(\%-w/w)}.100$$

The "initial weight" in the equation refers to the weight of the container and the wort prior to pitching the yeast. The weight used to calculate the "loss in weight" in the equation was the mass of the container measured daily, including its contents, while the "full weight" refers to the contents of the container on the day the yeast was pitched, which amounted to approximately 2000 g. In order to calculate the volume of CO_2, a density under normal conditions of 1.815 kg/m^3 was assumed.

3.2.2 Determining the cell count

Cell count of the pitching yeast: The amount of pitching yeast was determined using a cell counter (PeqLab) and verified with a Thoma cell counting chamber.

Analysis of the cells in suspension (up to day 2 of fermentation) and total cell count: So that the cell counts during fermentation could be accurately determined, the head pressure in the vessel had to be released very slowly. Using a 10 ml volumetric pipette mounted on a stand, 30 ml of green beer was removed from the middle of the column of liquid in each fermentor, in order to measure the number of cells in suspension. The green beer from the fermentor was homogenized immediately after removal using a magnetic stirrer prior to taking a sample and determining the total cell count.

3.2.3 Determining the surface charge

The surface charge corresponds to the equivalent point (eq) of a titrant per volume of yeast suspension (ml). In these trials, a solution of polydiallyldimethylammonium chloride (0.0001 N) was used. The surface charge was calculated using the following equation:

$$charge\left(\frac{eq}{cell}\right) = 0.0001N/ml\,\frac{titration\ volumen(ml)}{YC\left({cells}/{ml}\right)}$$

Preliminary tests showed that a linear correlation exists between the surface charge and the cell count. It follows that the more cells there are in suspension, the more titrant will be required.

The suspensions were prepared as follows: the required sample volume was centrifuged for 5 min at 5 °C and 750 g. After decanting the supernatant, the sediment was weighed, and 10 times its weight in tap water was added (1:10). 4 ml of the suspension was diluted to 100 ml and the titration was performed. Finally, the exact cell count was determined, so that the results could be correlated perciseely to the biomass contained.

3.2.4 Sensory analysis

After the beers were lagered, they were subject to sensory analysis by ten experienced, DLG-certified tasters at the Weihenstephan Research Center.

In addition to this evaluation according to the DLG scheme, specific flavors and aromas were also described. It was decided that at

least one third of the panelists must perceive the aroma before the aroma would be deemed significant in the sensory analysis.

4 Results

4.1 Reduction in extract concentration and CO_2 production

Since the yeast strains in this study were characterized with reference to the behavior of yeast strain TUM 34/70, the results for each individual parameter are presented with regard to their relative divergence from the results for the parameters exhibited by 34/70 ($|parameter|_{TUM-X} - |parameter|_{TUM-34/70}$). The chart below shows the extract difference measured during the fermentations conducted for this investigation. A positive deviation indicates a proportionally slower fermentation.

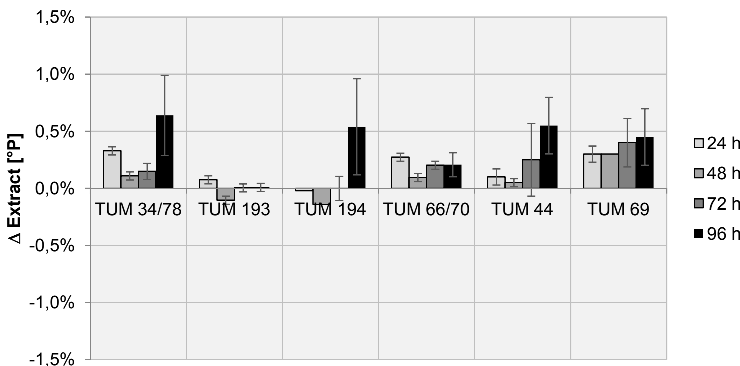

Fig. 2 Change in extract concentration compared to TUM 34/70 during primary fermentation

As shown in figure 2, the differences in the drop in the specific gravity are relatively small. The largest differences (approx. + 0.5% °P) are mostly observed at the end of fermentation. Nevertheless, it can be asserted that in general, TUM strains 34/78, 66/70 and 69 ferment the wort at a somewhat slower rate.

This is also reflected in the amount of CO_2 produced during fermentation. TUM 69 produced significantly less CO_2 than TUM 34/70.

Table 2 CO_2 production in relation to decline in extract concentration

Yeast strain	Increase, i.e. CO_2 production (% g/100 g extract)	R^2	% difference to TUM 34/70
Gay-Lussac	48.90	–	–
Balling	46.29	–	–
TUM 34/70	43.15	0.99661	–
TUM 34/78	41.24	0.99434	–4%
TUM 193	41.50	0.99429	–4%
TUM 194	40.88	0.98157	–5%
TUM 66/70	43.86	0.99719	2%
TUM 44	42.35	0.99708	–2%
TUM 69	42.29	0.99563	–2%

By contrast, TUM 193 generated somewhat more CO_2 than TUM 34/70. TUM 66/70 exhibited no difference during fermentation. TUM strains 194 and 44 produced comparatively more CO_2 at the beginning of fermentation, but this tapered off as fermentation progressed.

If the values for the CO_2 concentration in °P are plotted against those for the degree of attenuation during fermentation, linear correlations result for all yeast strains. The slopes and R^2 are provided in table 2 below. The slope represents the mass of CO_2 produced per unit of extract consumed.

4.2 Change in pH value and acidification capacity

Although a rapid drop in pH has a negative effect on foam stability and bitterness yield in general, a low pH value also serves to inhibit the growth of harmful organisms [13]. For this reason, an initial rapid drop in pH is considered desirable, as are lower pH values in the finished product. Figure 3 shows the differences in the pH values measured for the yeast strains investigated compared to TUM 34/70.

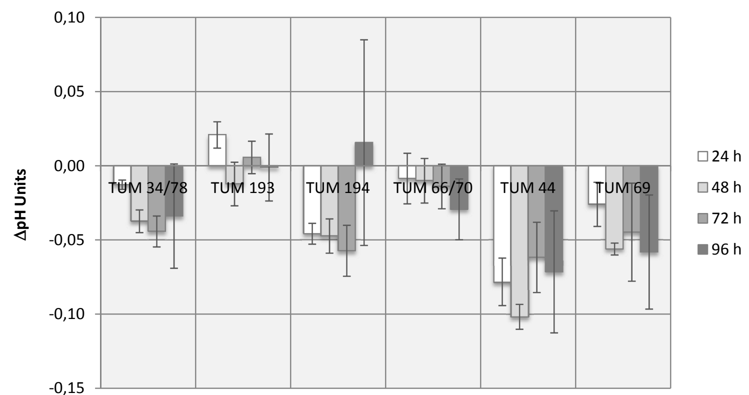

Fig. 3 pH measurements during fermentation compared to TUM 34/70

According to the chart above, TUM 44 exhibited the strongest capacity for acidification compared to other strains. TUM strains 34/78, 194 and 69 also displayed a tendency for producing lower pH values. No clearly perceptible differences were observed for TUM strains 193 and 66/70.

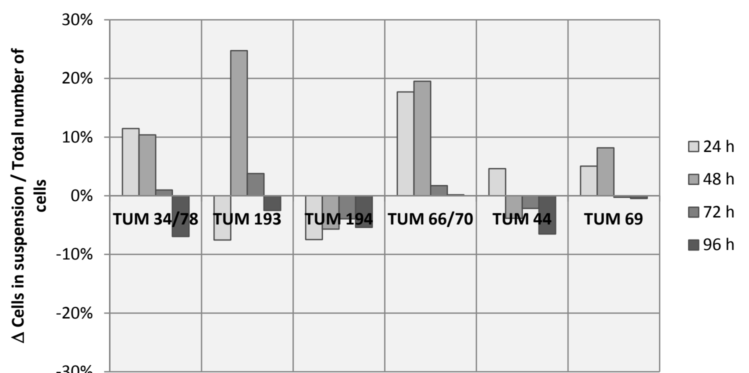

Fig. 4 Cells in suspension compared to TUM 34/70

4.3 Number of cells in suspension, yeast propagation and sedimentation behavior

Figure 4 gives the percentage of cells in suspension, divided by the total number of cells present in the fermentation systems. The 0 % axis corresponds to behavior identical to strain TUM 34/70.

As indicated in the figure above, TUM 66/70 remained in suspension markedly longer than the other yeast strains during the first two days of fermentation. In addition, TUM 34/78 exhibited a more "powdery" or non-flocculent behavior, followed by strain TUM 69. By contrast, TUM 194 sediments out more rapidly than strain TUM 34/70, while TUM 44 did not differ significantly from TUM 34/70 with regard to the parameters selected.

Visual observation of the sediment volume in the Imhoff cones, however, yielded no definitive results, because the standard deviation was very high.

4.4 Change in turbidity

The turbidity was also monitored over the course of fermentation. The initial turbidity values measured in the pitching wort were taken into account. Figure 5 shows the differences in turbidity compared to TUM 34/70.

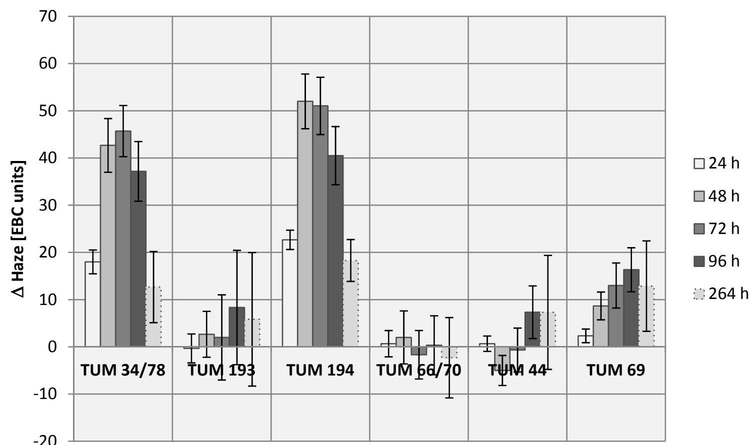

Fig. 5 Turbidity compared to TUM 34/70

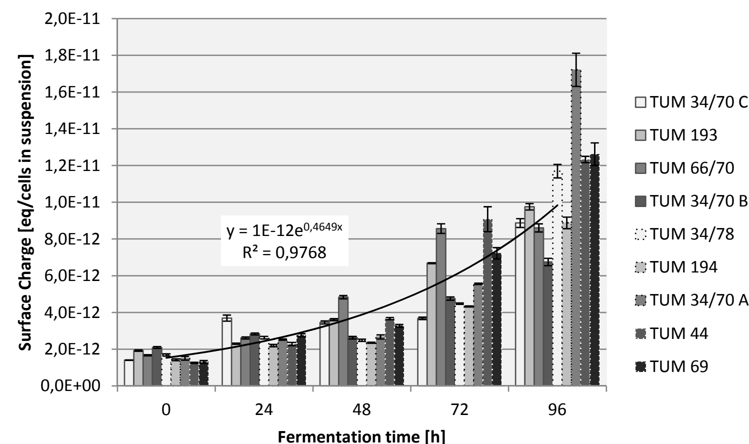

Fig. 6 Charge on the surface of yeast cells in suspension during fermentation

TUM strains 34/78 and 194 produce 10 % to 25 % more turbidity in green beer, followed by TUM 69 with about 5 % more turbidity. No significant differences were observed between TUM 34/70 and the TUM strains 193, 66/70 and 44.

4.5 Changes in the surface charge of yeast cells

Figure 6 depicts the change in the amount of charge on the cells in suspension for each yeast strain which is expressed in eq/cell. In this chart, the data from the three trials (A–C) are plotted individually and are not shown as a comparison to reference strain TUM 34/70.

As is evident from the graph, the charge on the cells in suspension increased over time for all strains and is therefore not strain-specific. A strong increase was recorded starting on the third day of fermentation. A peak was reached on the fourth day which was equivalent to 5–6 times the initial amount of charge present upon pitching.

Measurements of the surface charge on the sedimented yeast, however, did not indicate a definitive pattern. For this reason, a graphical representation of these data was not included in this publication.

4.5.1 SO_2 formation

Figure 7 shows the SO_2 content in the unfiltered beers after lagering compared with the reference strain TUM 34/70.

As indicated in figure 7 below, TUM 193 produces about 90 % more SO_2 than TUM 34/70 under the conditions present in the trial. TUM strains 66/70 and 44 form 21 % and 11 % more SO_2, respectively,

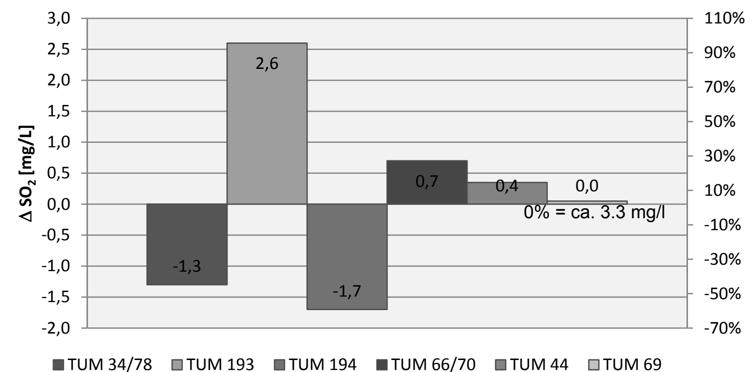

Fig. 7 SO_2 concentration in beer compared to TUM 34/70

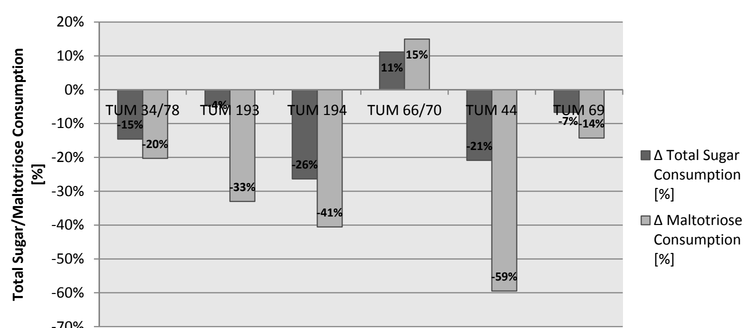

Fig. 8 Sugar uptake after maturation compared to TUM 34/70

whereas TUM 69 produces on average approximately the same amount of SO_2 as TUM 34/70. Comparatively, TUM strains 194 and 34/78 produce 54 % and 42 % less SO_2, respectively.

4.5.2 Sugar utilization

Strain-specific differences in sugar uptake, especially for maltotriose are described in the literature [15] [16]. The following chart shows the total sugar uptake, as well as the maltotriose consumption measured in beers after maturation and lagering.

Figure 8 shows that strain TUM 66/70 metabolizes 11 % more sugar on average during fermentation and maturation than does yeast strain TUM 34/70. All other yeast strains metabolize less sugar. The maltotriose consumption closely matches the total sugar consumption. With reference to TUM 34/70, only TUM strain 193 exhibits a significantly lower maltotriose metabolism (–33 %) compared to its total sugar consumption (4 %). TUM 44 utilized 59 % less maltotriose than TUM 34/70.

4.5.3 Diacetyl

In order to explore the degradation of diacetyl more in-depth, the diacetyl concentration was adjusted by means of an exponential function. An example is shown in figure 9, in which the total diacetyl concentration from all three trials measured for yeast strain TUM 34/70 is plotted against fermentation time.

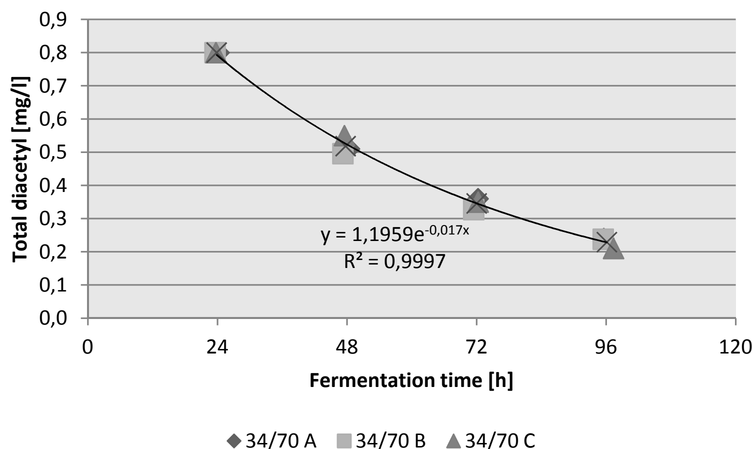

Fig. 9 Change in (total) diacetyl for TUM 34/70

The use of an exponential function reveals a general trend in the data gathered from the three trials with TUM strain 34/70 which is evident from the graph. This behavior was observed independent of the yeast strain utilized. Thus, the degradation of diacetyl can be described mathematically as follows:

Formula: Total diacetyl concentration [mg/l] = a.exp[–b.fermentation time(h)]

Table 3 summarizes the results gathered from trials using the following parameters (30 million yeast cells/ml, 15 °C, 0.5 bar of head pressure, 11.8 °P original gravity).

It is apparent from the calculation that all yeast strains require 6–7 days of fermentation to degrade the diacetyl and lower the concentration to less than 0.1 mg/l. Of the strains tested, only TUM 194 was similar to the reference strain TUM 34/70 with regard to the rate of diacetyl degradation. All other strains required longer to reduce the concentration of diacetyl (total diacetyl) to below the threshold value.

At the end of maturation and lagering, the total diacetyl content measured for almost all of the yeast strains tested was less than 0.10 mg/l. Only the beer samples produced using TUM 66/70 still possessed a concentration of 0.11 mg/l.

4.5.3.1 Amyl alcohols

The concentration of amyl alcohols present in beer fermented with other yeast strains is compared to TUM 34/70 in figure 10 and expressed as a percentage.

Based on the information in the chart, it is apparent that TUM yeast strains 34/78, 194 and 66/70 produced lower concentrations of amyl alcohols (2–9 mg/l) than TUM 34/70. TUM strains 193 and 44, however, produced 7–14 % (4–8 mg/l) more amyl alcohols in the finished product.

4.5.3.2 Ethyl acetate

Esters number among some of the most important aroma compounds in beer and thus have a significant impact on flavor. Figure 11 shows the difference in ethyl acetate produced by the yeast strains tested compared to TUM 34/70.

Table 3 A mathematical comparison of diacetyl degradation

Yeast strain	a	b	R^2	Time [d] calculated for diacetyl concentration to be reduced to 0.1 mg/l	Difference in time [h] required for TUM 34/70 to reduce diacetyl to 0.1 mg/l
TUM 34/70	1.1959	0.017	0.9997	6.0	–
TUM 34/78	0.7907	0.013	0.9733	6.5	+12
TUM 193	0.8079	0.014	0.9928	6.1	+2
TUM 194	0.8610	0.015	0.9848	6.0	+0
TUM 66/70	0.8479	0.013	0.9837	6.8	+19
TUM 44	1.2800	0.017	0.9781	6.3	+7
TUM 69	1.1753	0.016	0.9946	6.6	+14

Formula: Total diacetyl concentration [mg/l] = a.exp[–b.fermentation time(h)]

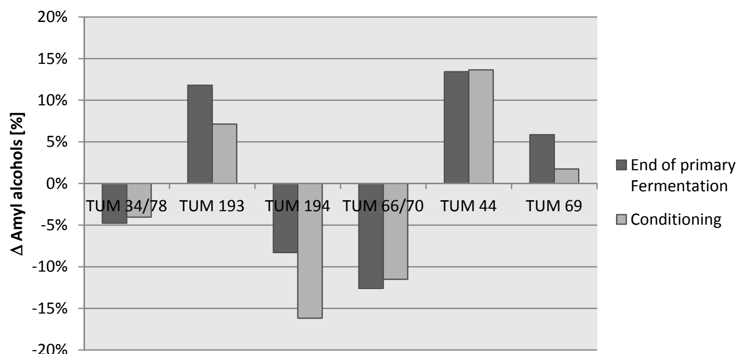

Fig. 10 Concentration of amyl alcohols compared to TUM 34/70

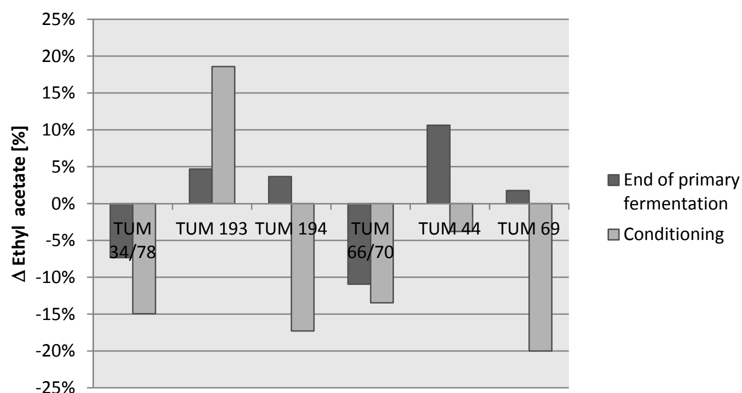

Fig. 11 Concentration of ethyl acetate compared to TUM 34/70

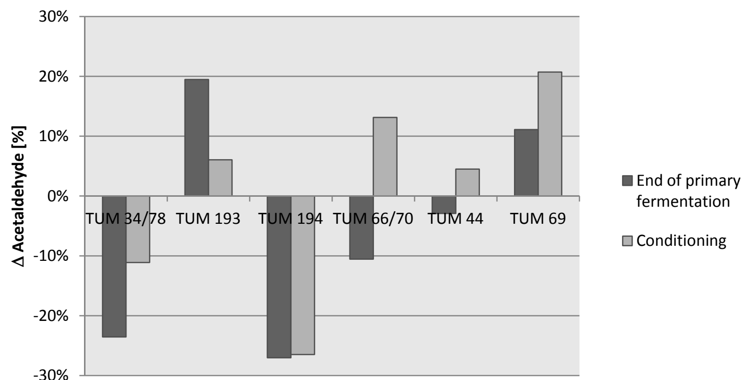

Fig. 12 Concentration of acetaldehyde compared with TUM strain 34/70

The illustration shows that TUM strains 34/78, 194 and 69 produced 13 to 20 % less ethyl acetaldehyde than TUM 34/70. TUM 193 proved to be the exception, producing a higher concentration of ethyl acetate, almost 20 % by comparison. Nevertheless, all ethyl acetate concentrations after lagering were significantly below the threshold value described in the literature.

4.5.3.3 Acetaldehyde

Acetaldehyde is an aroma compound typically associated with beer which has not yet sufficiently matured, or "green beer" as it is known. Figure 12 shows the difference in acetaldehyde concentrations produced by the other strains compared to the concentration for

Table 4 Amino acid uptake for all yeast strains during the first 24 h of fermentation in chronological order

193	34/70 A	66/70	34/78	34/70 B	194	44	34/70 C	69
Serine	Serine	Asparagine	Asparagine	Serine	Serine	Serine	Serine	Asparagine
Asparagine	Asparagine	Serine	Serine	Asparagine	Asparagine	Asparagine	Asparagine	Serine
Methionine	Lysine	Lysine	Threonine	Threonine	Threonine	Threonine	Threonine	Lysine
Lysine	Methionine	Methionine	Lysine	Lysine	Lysine	Lysine	Lysine	Threonine
Histidine	Leucine	Threonine	Leucine	Glutamine	Methionine	Leucine	Leucine	Aspartic acid
Glutamine	Histidine	Histidine	Methionine	Leucine	Leucine	Alanine	Histidine	Methionine
Threonine	Threonine	Leucine	Alanine	Methionine	Isoleucine	Histidine	Arginine	Leucine
Leucine	Glutamine	Glutamine	Isoleucine	Alanine	Glutamine	Methionine	Tyrosine	Histidine
Phenylala-nine	Alanine	Isoleucine	Glutamine	Isoleucine	Phenylala-nine	Arginine	Alanine	Isoleucine
Isoleucine	Isoleucine	Aspartic acid	Histidine	Histidine	Histidine	Glycine	Methionine	Phenylalanine
Alanine	Phenylala-nine	Phenylala-nine	Phenylala-nine	Phenylala-nine	Tyrosine	Tyrosine	Glycine	Glutamic acid
Aspartic acid	Tryptophan	Alanine	Tyrosine	Glutamic acid	Alanine	Isoleucine	Isoleucine	Alanine
Glycine	Aspartic acid	Valine	Valine	Aspartic acid	Valine	Tryptophan	Aspartic acid	Tyrosine
Valine	Glutamic acid	Tryptophan	Glutamic acid	Tyrosine	Glutamic acid	Aspartic acid	Valine	Arginine
Glutamic acid	Tyrosine	Glutamic acid	Tryptophan	Tryptophan	Tryptophan	Glutamine	Phenyl-ala-nine	Valine
Arginine	Valine	Tyrosine	Aspartic acid	Valine	Aspartic acid	Valine	Glutamic acid	Tryptophan
Tyrosine	Glycine	Glycine	Glycine	Arginine	Arginine	Phenyl-ala-nine	Glutamine	Glutamine
Tryptophan	Arginine	Arginine	Arginine	Glycine	Glycine	Glutamic acid	Tryptophan	Glycine

Group A = light gray, Group B = dark gray, Group C = no shading

TUM 34/70. The values shown in the graph reflect concentrations measured at the end of fermentation and the end of maturation.

After primary fermentation and lagering, the beers fermented with TUM 34/78 and TUM 194 contained less acetaldehyde than the reference sample. By contrast, beers fermented with TUM strains 69 and 193 possessed up to 20 % more acetaldehyde.

4.6 Amino acid uptake and utilization

As part of these trials, the individual amino acids were monitored over the course of fermentation, in order to determine whether differences in amino acid uptake and utilization are strain-specific. Figure 13 provides the sum of all the amino acids (FAN) evaluated in these trials.

As can be seen in the graph, fluctuations appeared over the course of the trials, which made a strain-specific comparison of yeasts during fermentation difficult. It is nevertheless evident from these data how slowly TUM 66/70 absorbs amino acids. The mean value curve represents an average FAN concentration for all of the trials. The curve shows a rapid drop in the FAN concentration of around 50 % in the first 24 h. Subsequently, the FAN remains relatively constant and then rises again in the lagered beer.

To better illustrate the strain-specific sequence of amino acid absorption, the results for the first 24 h are presented in table 4. The groups cited in the literature are indicated with shading.

Table 4 (see page 45) shows that the sequence of amino acid absorption is different for each strain. Nevertheless, clear guidelines for the amino acid uptake of each strain cannot be scientifically established based on these trials. Even for the reference strain 34/70, obvious differences are evident in each of the three series of trials.

The mean for amino acid uptake within the first 24 h is presented in figure 14.

As shown in figure 14, serine and asparagine were directly and most rapidly absorbed and metabolized by all of the yeast strains equally, followed by lysine and threonine. All four amino acids belong to group A. Afterwards, the other amino acids were consumed in the order indicated, though the grouping cited in the literature did not always apply to the results obtained in these trials [23].

It was notable, however, that one group of amino acids was depleted until the amino acids reached a certain level, as shown below, while a second group, although depleted, was subsequently excreted by the yeast cells.

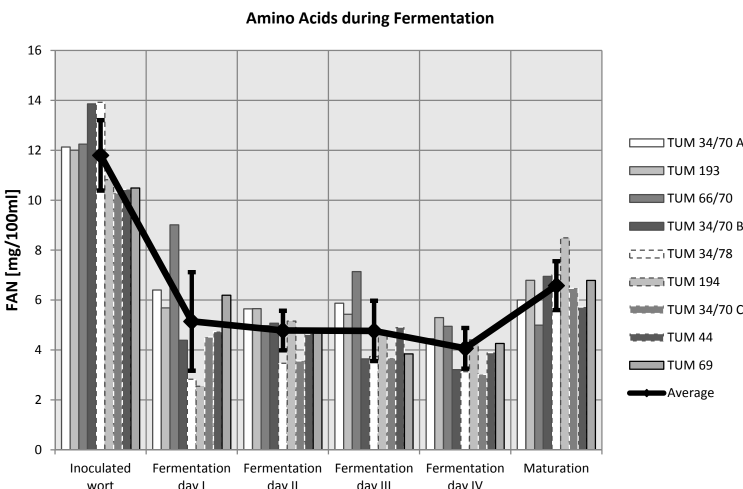

Fig. 13 FAN concentrations of all the yeast strains

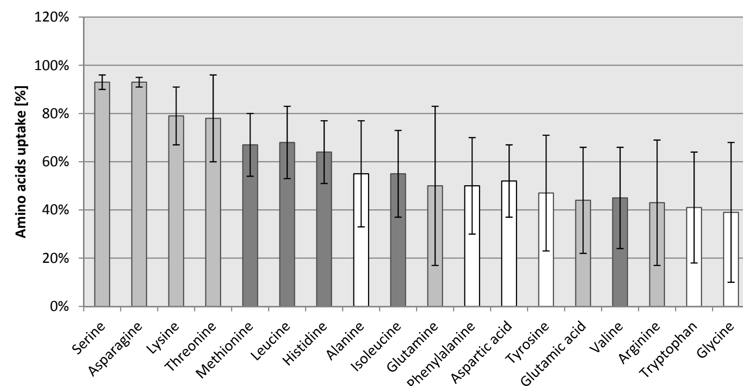

Fig. 14 Mean amino acid uptake during the first 24h of fermentation for all yeast strains

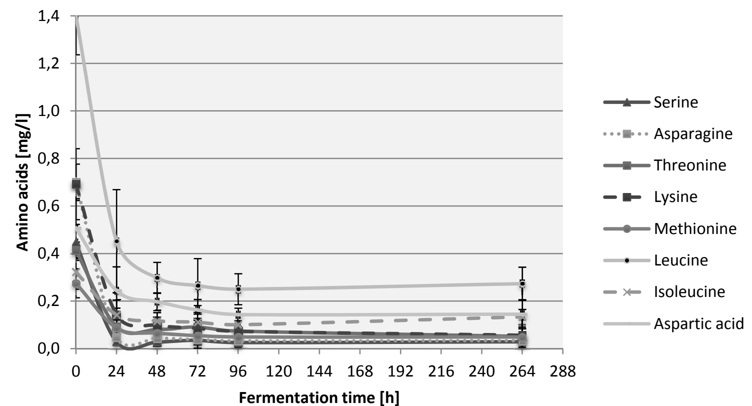

Fig. 15 Amino acids which are also not released during lagering

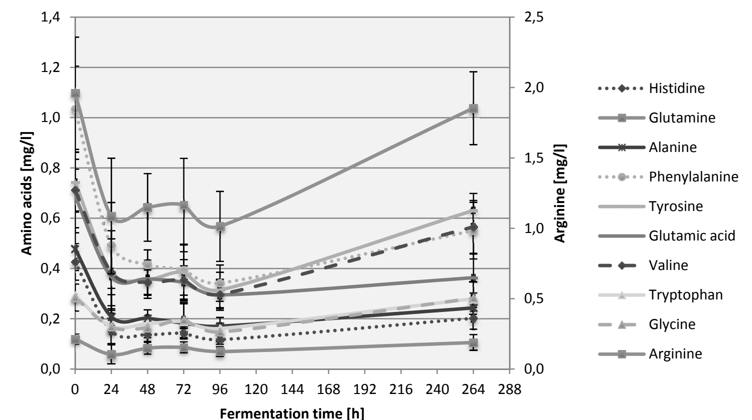

Fig. 16 Amino acids released into beer during lagering

If the average uptake of amino acids over a 24 h period is plotted against their molecular mass, different behaviors become apparent. In figure 17, the amino acids were divided according to their complexity into two groups: simple and complex. Simple amino acids are those in which a linear alkane is found in a side chain, which is the case with serine, threonine, leucine, isoleucine, aspartic acid, glutamine and glutamic acid. Complex amino acids are defined as those possessing aromatic structures or other elements in side chains, such as sulfur or additional NH_2 groups. These include asparagine, lysine, methionine, histidine, phenylalanine, arginine, tyrosine and tryptophan. Although the amino acids valine and alanine would be considered simple amino acids under this definition, the data indicate that they react differently, as shown below.

Figure 17 demonstrates that the uptake of amino acids generally decreases with increasing molecular weight (MW). This may be the result of a barrier to amino acid transport through the cell membrane. It was also observed that depending on their complexity, amino acids were taken up or utilized by the yeast cells at different rates. For example, asparagine (MW: 132.2) was metabolized approximately twice as rapidly as leucine (MW: 131.2), isoleucine (MW: 131.2) and aspartic acid (MW: 131.1), although they possess a similar molecular weight.

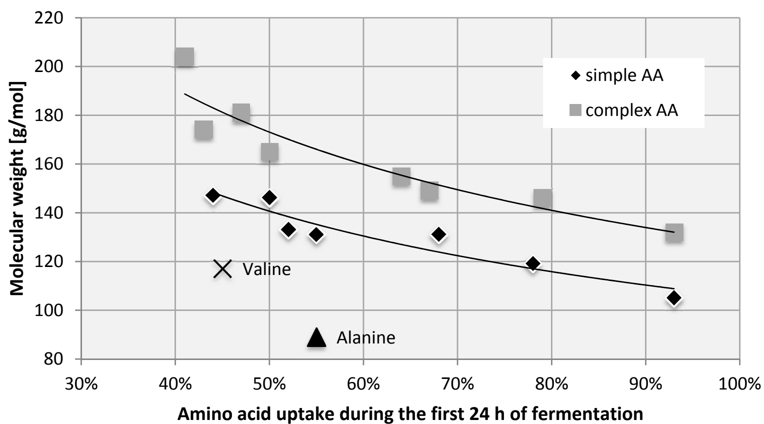

Fig. 17 Amino acid uptake over 24 h, based on molecular weight

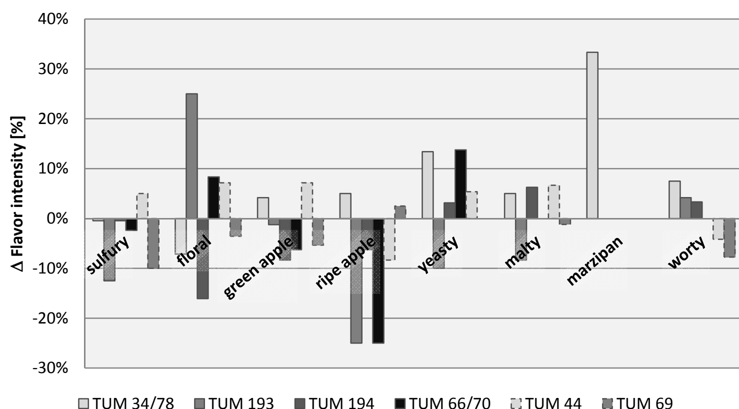

Fig. 18 Intensity of the flavors compared with TUM 34/70

4.7 Sensory analysis

Sensory analysis of the beers was conducted after maturation and lagering. Each was compared to the beer produced with TUM 34/70. Figure 18 indicates which differences were perceived by at least three of the ten trained tasters as significant.

The graph shows that the sulfur flavor in the beers prepared using TUM strains 193 and 69, according to the designated fermentation parameters, was found to be approximately 10 % less discernible, while this flavor was perceived as slightly more distinct (+5%) using strain TUM 44. TUM strains 34/78 and 194 produced slightly less floral beers. The mouthfeel was especially pronounced in the beer produced using TUM 193. Differences in the apple aroma were apparent, particularly with reference to the ripeness of the fruit. Thus, TUM strains 193 and 66/70 were found to produce beers with a less ripe apple aroma, while TUM strains 34/78 and 66/70 gave rise to beers with a more pronounced yeasty note, especially when compared to TUM 193. By contrast, little variation in the malt and wort notes was evident; however, with TUM 34/78, a much more prominent marzipan flavor was perceived.

4.8 Experimental synopsis

In these trials, differences between commonly used commercial strains of bottom fermenting yeast (TUM 34/78, TUM 193, TUM 194, TUM 66/70, TUM 44 and TUM 69 each compared to TUM 34/70) were investigated and described statistically. In order to obtain information relevant to conditions typical in commercial beer production, the lager beer wort (all-malt recipe at 11.8 °Plato) was fermented at 15 °C with at 0.5 bar of head pressure during primary fermentation (to simulate a 10 m fill level) and lagered at 2 °C. The yeast was always pitched at a concentration of 30 million cells/ml. In order to prevent the remaining contents of the fermentor from being influenced by daily sample collection, a system with 27 individual vessels was used. Using this system, samples for each yeast strain were taken from three fermentation vessels each day. In addition, a series of samples were collected to synchronously monitor lagering, turbidity and sedimentation.

The following table provides a qualitative summary of all results compared to the reference strain TUM 34/70. A more detailed description can be found in the section entitled Results.

5 Outlook and discussion

In order to establish the fermentation performance of a yeast strain, gravimetric determination of the amount of CO_2 production was found to be more precise compared with monitoring the change in extract using density determination. In addition, CO_2 formation proved to correlate exactly with the consumption of fermentable sugars. However, differentiation of "powdery" yeast from strongly flocculent strains based on gas formation as described by *Hoffmann* could not be confirmed in this study.

The yeast strains analyzed in this research showed different rates of maltotriose utilization, which is why this analysis could potentially be used for strain differentiation. However, *Hammond* and *Wenn*

Table 5 Behavior of the evaluated yeast strains compared to strain TUM 34/70

Fermentation characteristic	34/78	193	194	66/70	44	69
Fermentation performance						
– = no difference, ↓ = reduction (each approx. 5 %), ↑= increase (each approx. 5 %)						
Extract consumption	↓	–	↓↓	↑	↓↓	↓
Rate of CO_2 production	↓	↑	↑	–	–	↓
Amount of CO_2 produced	↓	↓	↓	↑	↓	↓
Initial rate of fermentation	↓	–	–	↓	–	↓
Total sugar utilization	↓↓	↓	↓↓↓	↑↑	↓↓↓	↓
Maltotriose utilization	↓	↓↓	↓↓	↑	↓↓↓	↓
pH reduction						
pH reduction	–	↓	↑	–	↑↑	↑
Capacity for acid production	↑	–	↑	–	↑↑	↑
Physical behavior of the yeast cells						
Cells in suspension	↑	–	↓	↑↑	–	↑
Sedimentation in Imhoff cone	–	–	–	–	↑	–
Turbidity	↑↑	–	↑↑↑	–	–	↑
Fermentation by–products						
Diacetyl	↓	–	–	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	–	–
Acetaldehyde	↓	↑	↓↓	–	–	↑
Esters	↓	↑	↓	↓	–	↓
Fusel alcohols	↓	↑	↓	↓	↑	–
Aroma impression						
Sulfury	–	↓↓	–	–	↑	↓
Floral	↓	↑↑↑	↓↓	↑	↑	–
Apple (green)	↑	–	↓	↑	↑	↓
Apple (ripe)	↑	↓↓↓	↓	↓↓↓	↓	–
Yeasty	↑↑	↓	–	↑↑	↑	–
Malty	↑	↑	↑	–	↑	–
Marzipan	↑↑↑	–	–	–	–	–
Worty	↑	–	–	–	–	↓

claim that the utilization of maltotriose increases with the number of times the yeast is harvested and repitched [17], which should be taken into account and confirmed.

Although the drop in pH also varies by yeast strain, the observations of Piendl could not be confirmed in these tests. Thus, no clear differences could be identified between non-flocculent and flocculent yeast strains.

Three further parameters were also examined during this study in order to characterize the sedimentation behavior of the yeast strains: the number of yeast cells in suspension (in conjunction with potentiometric titration), the sedimentation volume at atmospheric conditions (Imhoff cone) and the measurement of turbidity. All parameters were measured daily, however, no definitive correlation could be found. A possible explanation could be the differing pressure conditions during fermentation. In this study, however, for the first time [20], it could be readily demonstrated that the surface charge of the yeast cells in suspension increases during fermentation independent of yeast strain, whereas no conclusions could be drawn regarding the yeast, which had already sedimented out.

Findings in this study indicate that diacetyl degradation reaction is strain-specific. It was shown that it is possible to establish a mathematical relationship for predicting the diacetyl degradation of each yeast strain. In the future, this should become an integral part of the brewing process.

In most cases, esters and fusel alcohols exhibited a correlation with one another. Given an increase or decrease in the ester concentration (compared to strain TUM 34/70), a parallel increase or reduction in the concentration of fusel alcohol was observed. The ratio of esters to fusel alcohols has a significant effect on the sensory impression of the finished product [22].

Also this study revealed that stains TUM 34/78 and TUM 194, which produced more SO_2 than the other strains, produced less Acetaldehyde simultaniously. Wheather this correlation is systematically or a coincident needs to be checked in further researches.

Amino acid uptake by bottom fermenting yeast does not appear to be strain-specific and seems to also depend on molecular size and complexity. However, based on the findings obtained in this study, we agree with the recommendation made by *Stewart* et al. [19] that the amino acids methionine, leucine and isoleucine should be included in group A. Similarly, the transfer of phenylalanine to Group B would be appropriate. Nonetheless, it is not completely clear how the amino acids tyrosine and tryptophan should be classified.

In this study, it was shown that yeast strains can be differentiated using the methods relevant to industrial brewery operations presented here. Therefore, future research could be conducted to further characterize additional strains (top fermenting yeast and non-*Saccharomyces* strains).

Several analyses, such as determining sedimentation behavior in an Imhoff cone, seem to be obsolete, while others appear to provide interesting new insights; for example, preliminary experiments have shown that ale yeast in suspension exhibited a higher surface charge.

The system design also allows modifications to be carried out easily, so that different types of wort can be fermented under variable conditions, for instance, at different pressures and temperatures.

6 Summary

In this paper a reliable method is presented in order to charackterize yeast stains under practical conditiones. The usage of a reference organism, in this case: yeast TUM 34/70 enables the user to compare multible parameters, even if experiments are not conducted simultaneously. The study revealed that differences in bottom fermenting yeast strains can be observed (see: Experimental Synopsis).

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