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## Evaluation and Development of an alternative Analysis Method for rapid Determination of Yeast Vitality

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# Evaluation and Development of an alternative Analysis Method for rapid Determination of Yeast Vitality

**Brewer's yeast has a significant impact on the taste and quality of beer. It is thus necessary to obtain reliable and operationally useful information of the organisms' vitality.**
**Methods currently used are oftentimes very tedious and involve high expenditure. This research work investigates whether and to what extent it is possible to determine yeast vitality with less effort, using an automated laboratory device that records progression of gas formation online by measuring pressure build-up. To be able to make optimal use of the equipment, ideal test parameters such as test temperature, cell concentration, fermentation medium, headspace volume and system settings were identified and described in detail. Based on the parameters identified, direct comparisons with other established measurement methods for determining yeast vitality followed, such as the Fermentometer Method according to *Hlaváček* and ICP measurement. The method developed here was found to provide reliable, reproducible and comparable results that correlated directly with the other methods. Based on these findings, some industrial-scale tests were carried out in order to fully evaluate the potential of this analysis for process optimization.**

Descriptors: Yeast, yeast vitality, $CO_2$ evolution, pressure build-up, fermentative capacity, yeast concentration

## 1    Introduction

By determining the fermentative capacity or vitality of yeast, the physiological condition of yeast can be established (2).

Laboratory methods currently used in industry and research for establishing yeast vitality are oftentimes too laborious and cost intensive to be integrated in the dynamic production process of breweries. As a consequence, only viability i.e. the number of "dead" cells in the population is determined in day-to-day routine. Methods used in industry have also the disadvantage that they often do not directly measure the fermentative capacity of the culture but rather the function of individual metabolic mechanisms or intracellular components.

In literature, various methods have been described for determining yeast vitality using direct measurement of certain fermentation products. In addition to measuring the development of alcohol, sugar, energy and biomass contents in pilot fermentations, measurement of fermentation carbon dioxide formed during all metabolic processes of yeast is a simple and reliable method for evaluating fermentative performance. In research work done by Hlaváček, *Narziß*, *Back* and *Müller-Auffermann* et al., correlation between $CO_2$ evolution and yeast vitality has been proven without

any doubt. Today, mainly the values established by Hlaváček are regarded as an official guideline for assessing yeast vitality in this regard [2, p. 56].

To-date, all $CO_2$-based measurement methods are manual ones. This makes evaluation of the measurement results more difficult and may be a possible source of measurement errors. It was also observed that sufficiently visible $CO_2$ evolution can be recognized only in the log-phase of the yeast [2, p. 145]. The timeline of gas formation thus also plays a significant part in evaluating the lag-phase and log-phase in the yeast propagation cycle, a fact that had to be taken into account when developing an alternative method for determining yeast vitality. In this investigation, a fully automatic gas monitoring system (gas pressure monitoring/GPM system) was selected for measuring the kinetics of microbial metabolic processes. This system employs high-sensitivity pressure measurement in the mbar range, remote transmission of analysis data to a software program in real time and automatic entry of measurement data on a spreadsheet.

## 2    General Information

### 2.1    Yeast vitality

The term "yeast vitality" characterizes metabolic activities of yeast samples (e.g. speed of fermentation, acidification potential, metabolic activities, ATP content, the amount of intracellular reserve substances) obtained with very diverse analysis methods and their ability to survive stress situations [2, p. 13]. As shown in table 1, vitality measurement methods can be subdivided into the following three main groups according to Heggart [3, p. 409]:

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**Table 1    Subdivision of vitality methods in accordance with Heggart [3, p. 3]**

| Method based on: | Example |
|---|---|
| Metabolic activity | Vitality staining |
| | Microcalorimetry |
| | Reduction of vicinal diketones (VDK) |
| | Protease activity of yeast |
| | Magnesium ion release test (MRT) |
| | Specific oxygen uptake |
| | Acidification potential test |
| | Intracellular pH value (ICP) |
| Measurement of cellular components | Adenosine triphosphate (ATP) |
| | Adenylate energy charge (AEC) |
| | NADH (fluorometric) |
| | Glycogen und trehalose |
| | Sterols and unsaturated fatty acids |
| Fermentative capacity or glycolytic flow rate | Glycolytic flow rate |
| | $CO_2$ measurement |
| | Rapid fermentation |

When using fermentative analysis methods, Hlaváček defines yeast batches as vital that can release between 25 and 28 ml of $CO_2$ after 3 h when fermenting 10 % maltose solutions at 20 °C with about 100 million yeast cells/ml (0.15 g dry yeast content in 30 ml). Other authors such as Narziß agree that, in addition to volumetric measurement of carbon dioxide formed, pressure build-up is another route for evaluation of yeast vitality. According to Narziß, fermentative capacity can, e.g., be evaluated by measuring and analyzing the pressure situation arising after 30 min [7].

Müller-Auffermann published another analysis method in 2011 [5]. In this process, yeast vitality is determined based on the volume of fermentation carbon dioxide formed in Einhorn's fermentation tubes (see picture on the left) after a predetermined time. Hutzler has taken the lead in developing this method further meantime, with the objective of giving small and medium-sized brewers in particular a simple tool for evaluating the physiological condition of yeast (6).

The table below shows the test parameters to be implemented in this follow-on method:

**Table 2    Test parameters for analyzing yeast vitality using the *Einhorn's* Fermentometer according to Müller-Auffermann and Hutzler**

| | |
|---|---|
| **Fermentation substrate** | Maltose solution (10 % m/v) |
| **Sample preparation** | Centrifugation: 750 g, 5 min; Making up with water to 200 Mio. YC/ml |
| **Mixing ratio** | 6 ml yeast suspension 200 Mio.YC/ml + 14 ml maltose solution (10 %-m/v) |
| **Equilibration time** | 60 min at 28 °C (incubator) |
| **Incubation temperature** | 28 ºC |
| **Fermentation time** | max. 120 min |

A culture is also generally deemed to be vital when it breaks down 1 % extract within the first 24 h after pitching with 10–20 million YC/ml at 20 °C and/or when rapid final attenuation takes place down to a fermentable residual extract of 0.1 to 0.3 % within 4 to 5 days [2, p. 56].
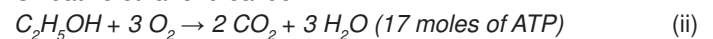
## 2.2    Formation and release of carbon dioxide

Brewer's yeast biochemical breakdown reactions generally serve the purpose of energy and biomass formation. Yeast respiration takes place under oxidative conditions. In this process, every 100 g of substrate are broken down to 55 g of carbon dioxide ($CO_2$) and 22.5 g of water ($H_2O$). This reaction (i) releases 38 mols of ATP. In the presence of oxygen in the medium and after depletion of all sugars that can be assimilated, yeast breaks down ethanol formed during fermentation oxidatively also to $CO_2$ and $H_2O$. This results in 17 mols of ATP (ii). These biochemical reactions are shown below [2, p. 171-173]:

*Oxidative sugar breakdown:*
$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \text{ (38 mols of ATP)} \qquad (i)$$
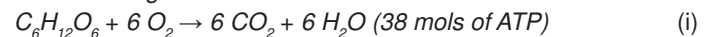
Oxidative ethanol breakdown:
$$C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O \text{ (17 moles of ATP)} \qquad (ii)$$

Alcoholic fermentation of yeast is another biochemical process in which every 100 g of substrate are broken down to 47 g of ethanol and 45 g of $CO_2$. However, this yields only 2 moles of ATP [1, p. 50]. This process is expressed by *Gay-Lussac's* classic equation:

*Fermentation:*
$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \text{ (2 moles of ATP)} \qquad (iii)$$
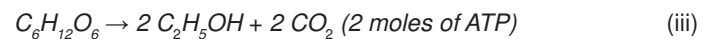
When analyzing the above three chemical breakdown reactions of brewer's yeast, it is obvious that carbon dioxide ($CO_2$) is the only main product formed in all metabolic processes for energy and biomass generation of the organism.

As the yeast plasma membrane has a high permeability for the nonpolar $CO_2$ gas, carbon dioxide formed inside the yeast cell is presumably released by simple diffusion through the cell membrane. This mechanism is the simplest form of mass transport and is based on *Fick's law*. Active transport using a carrier represents the second method of $CO_2$ release through the yeast cell membrane.

Formation of $CO_2$ is a function of fermentation intensity and, thus, yeast vitality, under the same conditions such as original gravity, available gas space volume, yeast pitching concentration and temperature. Analytic measurement of this parameter is thus useful for evaluation of the physiological condition of yeast.

## 3    Measurement Devices

### 3.1    Automatic monitoring system for fermentative gas evolution (GPM system)

In this research work, a gas monitoring system GPM (Gas Production Monitoring) from ANKOM[RF] was used. This is already in
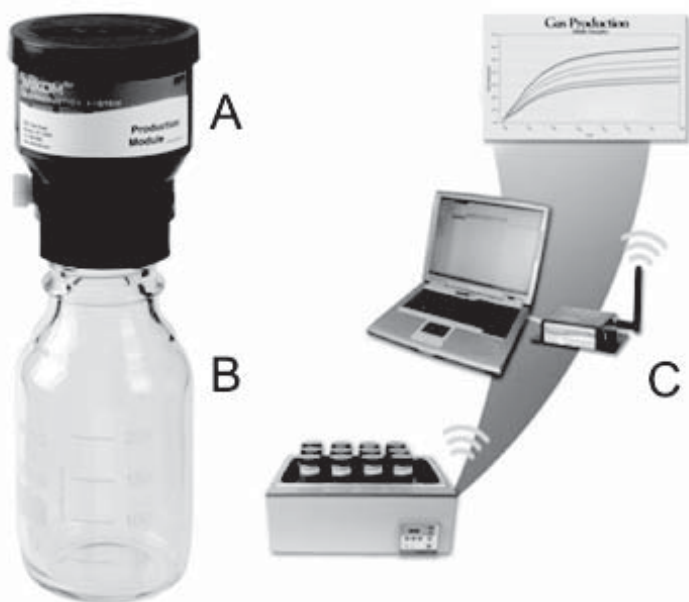
**Fig. 1    Arrangement and graph of the GPM system functional principle**

use for remote measurement and investigation of gas pressure build-up of microbial metabolic processes. Figure 1 shows the system arrangement.

In the test, measurement modules – A – are screwed onto commercially available laboratory flasks – B – containing the fermentation substrate pitched with the yeast to be analyzed. Each measurement module can be individually adjusted remotely using a controller. It contains a high-sensitivity pressure indicator that measures data in the mbar range and transfers such data in real time remotely to a computer. The pressure release valves can also be adjusted by the system control software as required. Flasks – B – are available in different sizes and designs, allowing high flexibility. Up to 50 measurement modules can be connected simultaneously via a network – C –. The reactors are preferentially tempered in a water bath. The frequency of data recording and gas pressure release can be selected as required. The data interface is another important performance feature, it can be linked to a spreadsheet for graphic display and statistical evaluation.

### 3.2    Fermentometer according to Hlaváček

Hlaváček, F. [4, p. 62] developed a process for determining yeast vitality at the beginning of the 1960's. This process was based on the $CO_2$ volume produced during fermentation. Various "Fermentometers" were designed, provided with burettes for measuring $CO_2$-volume. The picture on the left shows the arrangement of the Fermentometer according to Hlaváček. This method according to Hlaváček is the only such method commonly used to-date (2).

The Fermentometer design incorporates an Erlenmeyer flask – A – with



a net volume of 50 ml as reaction space. It is closed with a rubber plug – B –. A short glass pipe is inserted in it. Carbon dioxide formed during fermentation escapes into the measurement burette – D – via a three-way cock – C –. The lower part of the measuring burette – D – is connected to the equalizing vessel – F – by means of a rubber hose – E –. The burette and the equalizing vessel are filled with a 20% NaCl solution previously acidified with phosphoric acid to pH 1. Carbon dioxide is practically not absorbed in this solution [4, p. 63]. The picture on the left illustrates the Fermentometer setup.

For proper measurement, a yeast cell concentration containing about 100 million YC/ml (0.15 g of dry yeast content in 30 ml) at 20 °C should be used. Under the conditions described above, vital yeast batches should lead to the evolution of the following quantities of carbon dioxide as a function of fermentation substrate:

**Table 3    Carbon dioxide volume resulting from yeast batches of good vitality in the Fermentometer according to Hlaváček [4, p. 65]**

| Time (h) | $CO_2$ volume when using a 10 % maltose solution (ml) | $CO_2$ when using a 10 % saccharose solution (ml) |
|---|---|---|
| 1 | 5 to 8 | 3 to 5 |
| 2 | 15 to 18 | 9 to 11 |
| 3 | 25 to 28 | 24 to 26 |

### 3.3    Intracellular pH value

Hydrogen ion concentration [$H^+$] has both a direct as well as an indirect influence on the yeast cell. A low pH value (high [$H^+$] concentration) has a direct toxic effect on the yeast cell. The pH value also has an indirect effect as it influences the state of dissociation of nutrient substances and products of metabolism and, thus, the nutrient substance uptake rate and toxicity of dissolved substances. Medium pH has no direct influence on the inner yeast cell pH. As the intracellular pH value (ICP) has an influence on the activity of the enzymes of glycolysis and gluconeogenesis, it thus determines the vitality of metabolic performance of the yeast. The ICP of the yeast cell is controlled by the Plasma-Membrane-ATPase enzyme [10, p. 1148-1151].

Based on limit values determined by the Chair of Brewing Science and Beverage Technology of the Technische Universität Weihenstephan, yeast cells with intracellular pH values between 5.8 and 6.6 are capable of a high fermentative rate and a very good propagation performance during the brewing process. However, the yeast cell ICP changes during fermentation. It increases with fermentation activity (highest ICP during main fermentation in the high krausen phase) and gets reduced slowly again until final attenuation is reached. Lower pH values between 5.4 and 5.8 are considered as being average to good whereas pH values below 5.4 are considered to be unacceptable. These values are currently being applied in the brewing industry and are hence worldwide accepted for the evaluation oft he yeast vitality. However according to the values of the original author *Schneeberger* [10], the physiological condition of a yeast population can be evaluated based on the ICP values measured, using the following table.
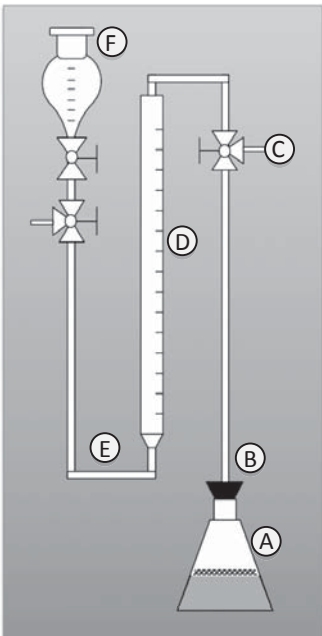
**Table 4     Indicator values of intracellular pH of beer yeast in accordance with Schneeberger [2, p. 162]**

| ICP value | Physiological condition |
|---|---|
| > 6.10 | very good |
| ≥ 5.70...≤ 6.10 | average |
| < 5,7 | poor |

## 4     Test Conditions

Before starting the tests, the conditions as set out below were specified for the method to be developed.

A. The method should provide reliable results within about 2 hours to keep pace with lab routine.
B. It should be possible to use quite a low yeast cell concentration in the tests so that low-yeast samples can also be examined.
C. Ideally, test conditions should be as close as possible to actual fermentation conditions in a brewery.
D. And, naturally, results should be similar to those of established methods.

### 4.1     General test conditions

In these investigations, thick slurry low-fermenting harvested yeast (always third cycle) of the TUM 34/70 strain was always used as this strain is often referred to as a reference strain in literature and strain 34/70 is regarded as the best researched brewer's yeast strain. Comparative tests, e.g. with established analysis methods, were always carried out with the same yeast simultaneously. All measurements were done at least three times. The results also always showed the confidence intervals calculated and/or the confidence interval was plotted in the abscissa.

### 4.2     Selection of a suitable nutrient medium

When developing his analysis method for determining yeast vitality, Hlaváček found differences when using saccharose or maltose solutions. This was taken into account in the current investigations and different fermentation substrates such as bottom-fermenting wort and a saccharose solution were tested.

### 4.3     Identifying the test volume in the GPM system

The gas theory equation describes the relationship between gas pressure and headspace volume. The smaller the headspace of the fermentation vessel, the faster pressure goes up in a closed system [9]. As test duration was a limiting factor, a sample quantity was specified that approximately corresponded to the fill height ratio of a cylindroconical tank of 60 to 80 % of the overall tank height. Using this fill height foaming over was also prevented.

### 4.4     Determining yeast cell concentrations and fermentation temperatures

The following method was selected in order to measure and determine yeast cell concentrations in the suspensions:

1. In order to be able to use the most vital yeast possible, harvested yeast from the third cycle (TUM 34/70) was transferred into sterile 5 l flasks from cylinderconical tanks of a brewery.
2. After yeast withdrawal and before cold storage at 4 °C, fermentation tests were carried out immediately with the freshly cropped yeast batch. This test was always used as a reference sample.
3. About 1 g of thick slurry yeast was placed in 100 ml measurement flasks and the contents were made up with water to the calibration line.
4. The yeast cell concentration of the yeast suspension previously diluted was then measured using a Cellometer® Cell Counter in accordance with the standard instructions of the manufacturer.
5. The following equation was used for preparing the master solution:

| **Weight (g)** | **Yeast suspension (ml)** | **Yeast cells (million YC/ml)** |
|---|---|---|
| $M_o$ (×) | $V_s$ (÷) | $YCC_s$ (÷) |
| $M_m$ (?) | | $YCC_m$ (×) |

$$M_m = \frac{M_o \cdot V_m \cdot YCC_m}{V_s \cdot YCC_s} \qquad \text{(I)}$$

| $M_m$ | = | Thick slurry yeast weight required for the yeast master solution (g) |
|---|---|---|
| $M_o$ | = | Weight as weighed for the dilution in the 100 ml measurement flask (g) |
| $V_m$ | = | Volume of measurement flask for the yeast master solution (ml) |
| $YCC_m$ | = | Yeast cell concentration of the yeast master solution required (million YC/ml) |
| $V_s$ | = | Volume of measurement flask for yeast suspension (ml) |
| $YCC_s$ | = | Yeast cell concentration of yeast suspension (million YC/ml) |

Naturally, it was taken for granted that the concentration of the yeast master solution should be equal to or higher than the pitched yeast cell concentration to be set.

6. In order to delay start of fermentation, the dilution medium, i.e. the fermentation substrate, was tempered down to 15 °C.
7. This was followed by weighing the thick slurry yeast quantity required for the master solution (Mm) and by filling the measurement flask with suitable cold medium (15 °C) to the calibration line (Vm).
8. Following pitching, samples were processed rapidly and pitching times were noted individually.
9. The yeast cell concentration of the yeast master solution was subsequently verified again using the Cellometer® Cell Counter.
10. In order to pitch with the exact yeast cell concentration specified, the yeast master solution was diluted down to the yeast cell concentration required in the ANKOM$^{RF}$ flasks by adding the particular nutrient medium. The following mass balance equations were used for this process:

$$VM_m [M_m] + V_w [W] = V_p [P] \qquad \text{(II)}$$

$$VM_m = \frac{V_P [P]}{[M_m]}$$

$VM_m$ = Volume of yeast master solution (ml)

$M_m$ = Yeast cell concentration of master solution (million YC/ml)

$V_W$ = Wort volume and/or volumes of fermentation media (ml)

$W$ = Yeast cell concentration of wort (million YC/ml). W = 0 million YC/ml

$V_P$ = Test volume (ml). $V_P$= 200 ml

$P$ = Pitched yeast cell concentration of test (million YC/ml)

$$V_W = V_P - V_{Mm} \qquad (III)$$

In addition to varying the yeast cell concentrations used, fermentation temperatures were also varied, as shown below. In order to do so, different water bath temperatures were used.
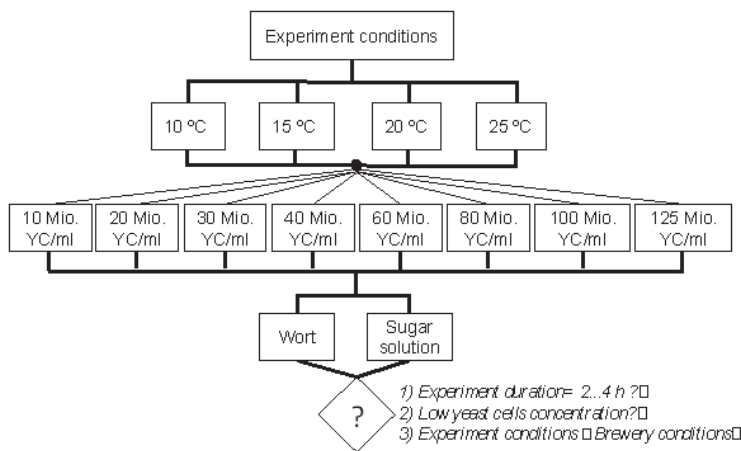


**Fig. 2    Schematic representation of test plan to identify optimum test conditions**

# 5    Results

## 5.1    Developing an analysis method

As no official method exists for investigating fermentation activity/vitality of brewer's yeasts with the GPM system, it was initially necessary to decide on suitable parameters.

Defining measurement volume

First of all, the objective of measurements had to be specified, taking account of previously defined conditions, in particular in terms of analysis duration. It was found in all pre-tests that, independently of substrate used, varying cell concentrations and fermentation temperatures always resulted in a certain shape of curve as shown in figure 3 by way of an example.

As shown in figure 3, serving as an example for the conditions of the test at 25 °C combined with 65 million YC/ml, pressure build-up is always exponential, with delay and slope – as will be shown later – changing in line with the condition of the yeast. It was found in all tests that exponential pressure build-up arises as of a gauge pressure of about 0.8 bar, independently of the parameters set (illustrated by the dotted trend lines).
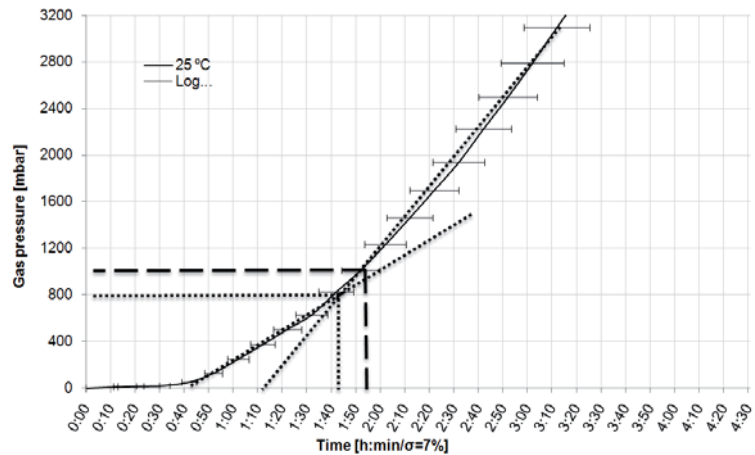


**Fig. 3    Average pressure build-up during several fermentation tests at a fermentation temperature of 25 °C**

In order to ensure that pressure build-up always has an exponential relationship to the evaluation period and that, the analysis can be fitted within a period of about 2 hours, it was specified for all subsequent tests that the **time required until a pressure of 1 bar had been built up (mathematically) in the system** had to be observed in order to assess yeast vitality (dashed line in Fig. 3).

Defining fermentation medium

In order to define test conditions, it is advisable to work with fermentation substrates that have consistent properties to ensure reproducibility and comparability.

However, defined fermentation substrates have the disadvantage that microorganisms might behave differently in an industrial environment as compared to the "artificial" conditions. In view of such differences, tests were carried out using fresh yeast and yeast stressed by warm storage in different media, as shown in figure 4.
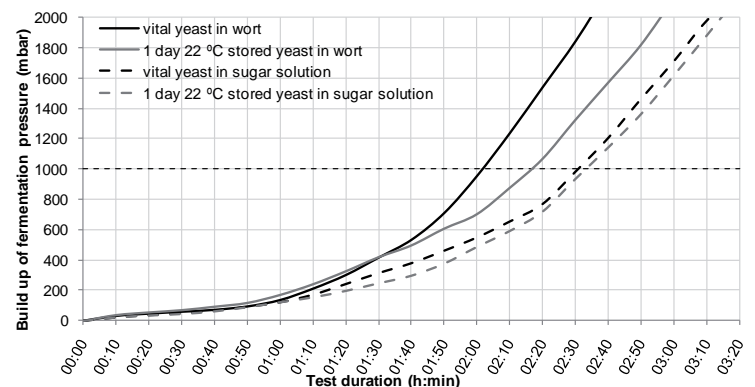


**Fig. 4    Pressure build-up of stressed and non-stressed yeast in different media**

Figure 4 shows that pressure build-up to the target pressure of 1 bar proceeds more rapidly in bottom-fermenting wort with 12 °Plato than in a pure saccharose solution. In addition, the differences between stressed and non-stressed yeast seem to be better detectable. This can presumably be attributed to the fact that saccharose molecules, during the preparation of the solution, is partly being degraded to glucose und fructose and these molecules, for example, enter the yeast cells by way of simple diffusion [2], thus reducing the sensitivity of the analysis developed [4]. As the objective here was to develop an operationally useful, practicable, simple and reproducible method reflecting conditions in breweries,

**bottom-fermenting lager beer wort with 12 °Plato** was ultimately chosen as fermentation substrate in all tests described (see Fig. 4).

## Defining fermentation temperature

Figure 5 shows the influence of test temperature and yeast cell concentration on gas generated and/or fermentation. However, results of the 10 °C and 15 °C tests are not shown graphically here because the time required to reach a pressure of 1 bar was too long to be acceptable in a daily routine in a laboratory, even when using high cell concentrations. Moreover, the confidence intervals are not shown explicitly in order to have a better overall view and understanding. However, confidence intervals were on average around 7 % (based on time).
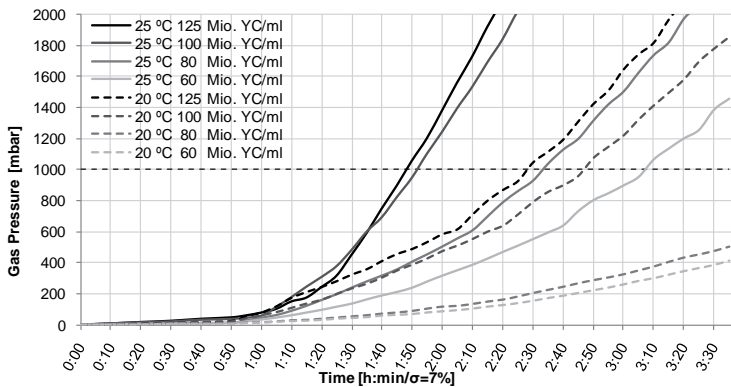


**Fig. 5    Gas pressure build-up at 25 °C and 20 °C and different yeast cell concentrations**

Figure 5 shows that the rate of gas pressure build-up and/or fermentative activity of the yeast batch goes up with increasing fermentation temperature and yeast cell concentration. As a result, a test temperature of **25 °C** was selected for all further tests, this is slightly below the temperature optimum of 26.8 °C for bottom-fermenting brewer's yeast [2].

## Defining pitching cell concentration

When running the tests at a temperature of 25 °C as previously specified, it was necessary to select the ideal cell concentration for subsequent tests. As mentioned in the specifications, this should be as low as possible. Nevertheless, pressure of 1 bar should be reached within a period of 2 to 3 hours. Test results are shown in figure 6 below.
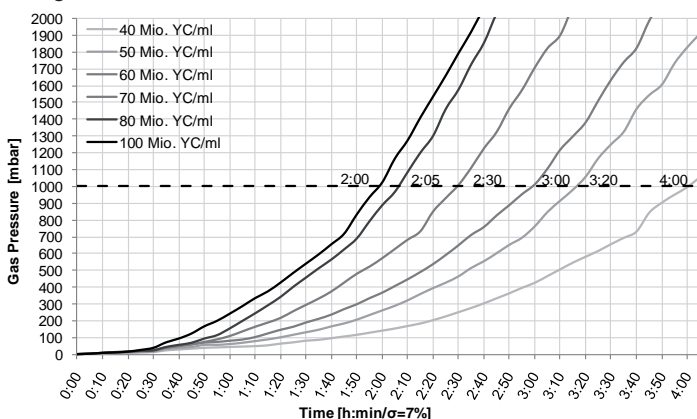


**Fig 6    Pressure build-up at 25 °C and different yeast cell concentrations**

As shown in figure 6, a shorter lag-phase as well as a more rapid start of fermentation set in with increasing pitched yeast cell concentrations. However, no linear relationship was obviously found between the time required to reach pressure of 1 bar and a uniform increase in yeast cell concentration (YCC). This means that the time difference until reaching target pressure becomes shorter with increasing YCC. In order to investigate this finding in more detail, figure 7 is a plot of times that were needed in order to build up pressure of 1 bar in the analysis flasks, each as a function of YCC.
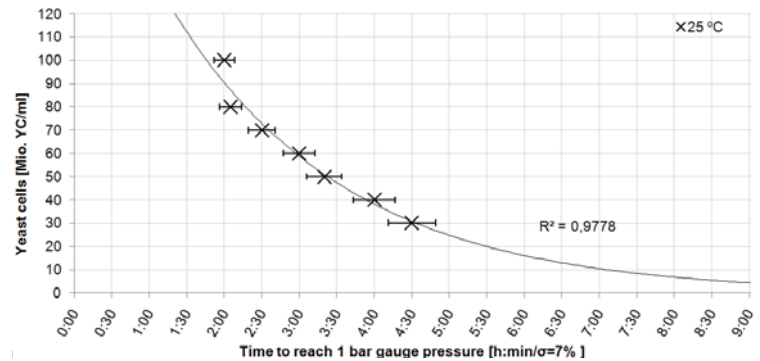


**Fig. 7    Time until reaching pressure of 1 bar at 25 °C as a function of YCC**

Figure 7 shows that pressure of 1 bar selected for evaluation is reached more rapidly with increasing YCC and that a more pronounced exponential curve arises. With cell concentrations over 80 million YCC/ml, it is no longer possible to clearly identify any differences in time to reach pressure of 1 bar.

Based on the results shown in figures 6 and 7, a YCC of **65 million YC/ml** was selected for the analysis method so that further tests with the shortest test durations possible and low YCC could be carried out.

## Summary of test conditions evaluated

The table below summarised test conditions specified in order to determine yeast vitality.

**Table 5    Test conditions for carrying out the analysis**

| Volume (ml) | 200 | wort and yeast |
|---|---|---|
| Temperature (°C) | 25 | in a water bath or refrigerator |
| YC (million per ml) | 65 | here: harvested yeast TUM 34/70 |
| Fermentation media | 12 °Plato wort | here: Weihenstephan „Original" lager beer wort |
| Goal (h : min) | time to reach 1 bar $_{ep}$ | exponential $CO_2$ formation |
| YCA to Headspace ratio | 118.18 million YC/ml headspace | adjustment if different reactor sizes are used |

## Statistical assessment of method at different temperatures

In order to examine whether the analysis method was subject to errors under the conditions mentioned, tests were repeated six times subsequently. In parallel, a series of measurements at cold

temperatures was carried out to assess dependencies. Based on these results, mean value, standard deviation, variance and confidence interval were calculated. In terms of confidence interval, a distribution factor of 2.3534 taken from the T-table was used, corresponding to a coefficient of determination of 95 % [8, p.214].
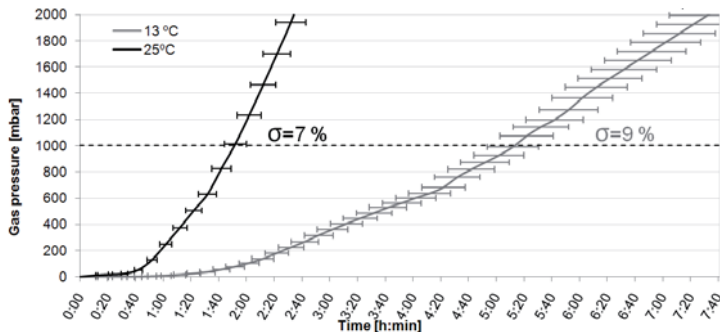


**Fig. 8**     Pressure build-up and confidence interval in testing vital yeast at 13 °C and 25 °C

As shown in figure 8, the confidence interval is (+/–) 7 % at the intended test conditions of 25 °C and 1 bar. If lower fermentation temperatures are selected, the confidence interval goes up to (+/–) 9 %.

Based on these findings, tests can be evaluated much better and follow-on tests could be carried out in triplicate so as to be statistically relevant. This in turn facilitates laboratory operations, raising precision in daily measurements.

### 5.2    Correlation between analysis method developed and established vitality measurement methods

Correlation with Hlaváček vitality measurement

Yeast vitality determination by establishing $CO_2$ evolution according to Hlaváček is an officially recognized analysis method for evaluating fermentative power of yeast batches [2, p. 56]. The method developed here is also similar to the Hlaváček measurement principle as both methods measure gas evolved during fermentation. In the Hlaváček method, gas formation is analyzed based on displacement of the liquid volume whereas, in the method described here, the resulting pressure is determined in a closed fermentation system. The correlations between the methods are shown in figures 9 and 10.
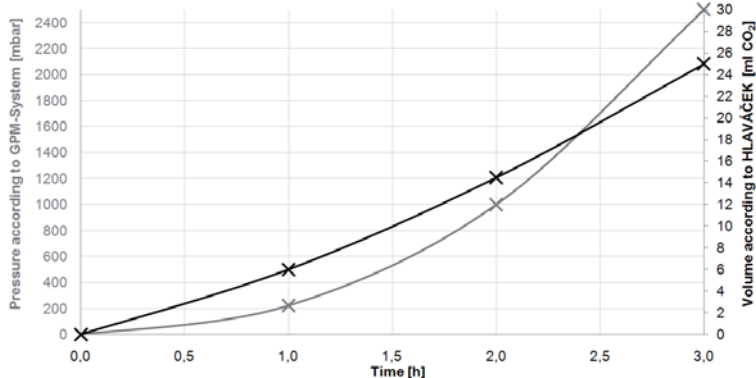


**Fig. 9**     Correlation between yeast vitality analysis according to Hlaváček and the method described here

As shown in figure 9, the curve according to the Hlaváček method has a linear form whereas the curve according to the GPM method described here has an exponential shape. The method described here seems to have the advantage that a delay in start of fermentation (lag-phase) can be captured more precisely.

Based on the Hlaváček evaluation criteria, yeast used in this test is regarded as being vital.

To be able to assess the significance of the correlation, the direct relationship between the two methods is shown graphically in figure 10.
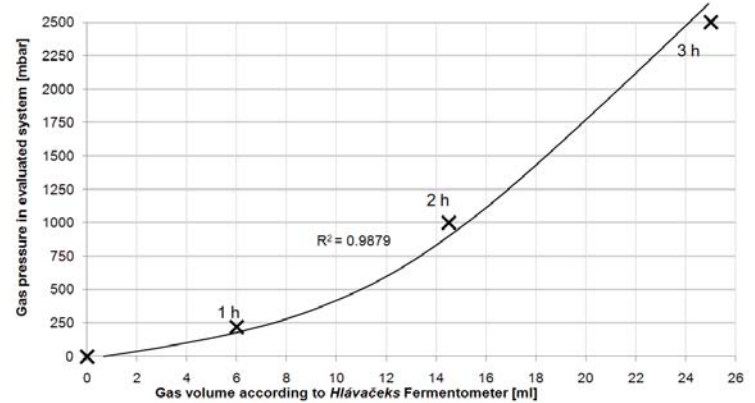


**Fig. 10**    Correlation between the method according to Hlaváček and the method described here

As confirmed by the high coefficient of determination of 0.99, the two methods correlate, i.e. gas volume measurement (according to Hlaváček) and gas pressure method, in large measure under the test conditions previously developed for this method.

Correlation with measurement of intracellular pH value (ICP)

For the purpose of validation, further comparative tests were carried out, including parallel measurement of the intracellular pH value. Fresh harvested yeast (TUM 34/70) was stored, without pressure, at various temperatures in several tests (storage at 15 °C is shown here by way of example) and vitality was determined after specified time intervals.
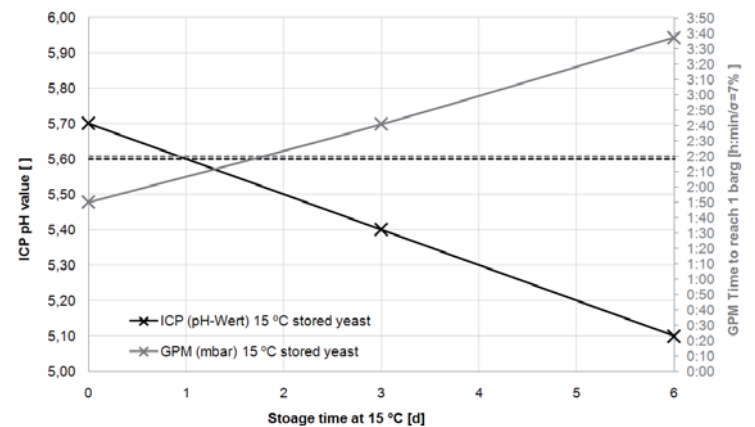


**Fig. 11**    Comparison between ICP analysis and GPM method developed for yeast stored at 15 °C

Figure 11 shows an inverse proportional relationship between the two analysis methods, independently of storage temperature. It can

be seen that fermentative capacity drops with decreasing intracellular pH in the course of storage but that the time until a pressure of 1 bar is reached goes up simultaneously and proportionally.

These curves are compared directly with one another in figure 12 in order to be able to evaluate correlations more precisely.
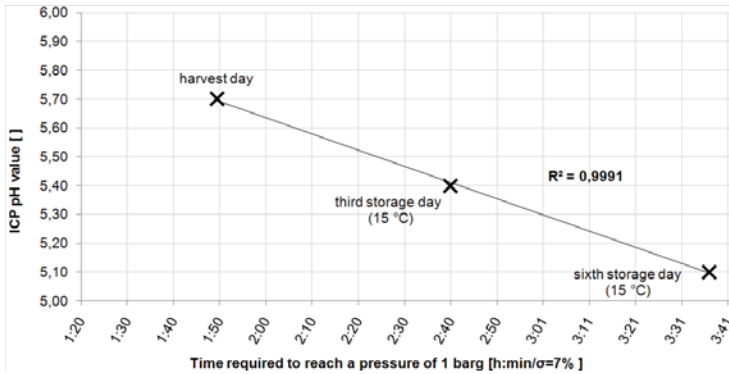


**Fig. 12     Correlation between ICP measurement and the analysis described here**

It is obvious that this results in an almost ideal linear correlation between the two analysis methods, with a coefficient of determination of 0.99. The intracellular pH of the yeast goes down with increasing yeast storage time whereas a pressure of 1 bar required for evaluation takes longer and longer to reach.

### 5.3     Determining benchmark and limit values for the method described here

The outcome of the comparative tests between the vitality determination method presented here and the established methods shows that the physiological condition of yeast is captured in large measure to almost the same extent in all methods tested.

Prior to specifying assessment standards also for this method, it was necessary to include yeast propagation in the parameters selected. For this purpose, a fresh vital yeast crop (ICP value: 5.6) was analyzed while, this time, cell concentrations from reference systems were measured in parallel at certain time intervals. The results of this test are shown graphically with the accompanying confidence intervals in figure 13.
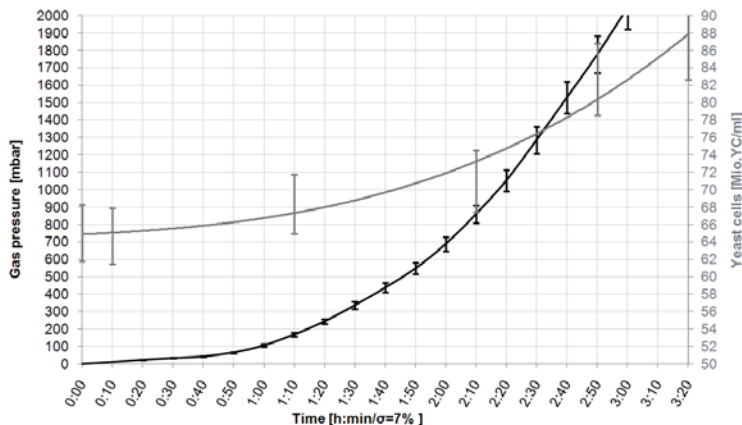


**Fig. 13     Relationship between $CO_2$ evolution and yeast propagation**

Figure 13 shows the relation between yeast multiplication and $CO_2$ evolution in the closed system. At a calculated threshold of 0,8 bar pressure, an exponential pressure increase occurs, parallel with the increase of biomass. This leads to the conclusion that the duration of the lag-phase can also be assessed with the method described here, as the latency time until the gas production starts rigorously. This test also shows that vital yeast is just at the very beginning of its exponential cell growth phase when 1 bar pressure is reached in the system and the cellular activity is at maximum, which means that all analysis parameters selected seem to be suitable for the intended purpose.

Based on the results of all tests, the following benchmark and limit values could be specified for the evaluation.

**Table 6     Benchmark values of GPM method and their significance for fermentative capacity**

| **Vitality (Fermentative Capacity)** | | | |
|---|---|---|---|
| **Method** | **Good** | **Sufficient** | **Insufficient** |
| ICP Value | ≥ 5.8 | ≥ 5.4...< 5.8 | < 5.4 |
| Müller-Auffermann/ Hutzler: Einhorn's Fermenter Method | ≤ 10 ml $CO_2$ in 80 min | ≤ 10 ml $CO_2$ in 80 min | > 10 ml $CO_2$ in 120 min |
| Hlaváček: (ml $CO_2$) | ≥ 25 after 3 hours | Not defined by author | < 25 after 3 hours |
| Müller-Auffermann/ Silva: GMP Method | < 2:20 | ≥ 2:20...≤ 2:40 | > 2:40 |

Table 6 shows the evaluation in terms of the vitality of a yeast batch in accordance with the various established methods and the analysis method developed here. It should be borne in mind that the evaluation criteria of the method presented here should be adapted individually to reflect the particular operational situation. If, e. g., another yeast strain or beer wort is used, it has to be decided in each instance how to go about arriving at an evaluation. The correlation with existing established methods can be used in order to adequately define the parameters, as has been done here.

The above-specified benchmark values are thus generally applicable only in gas pressure build-up analyses using a yeast cell concentration of 65 million yeast cells per sample volume in a lager beer wort medium with 12 °P, at a test temperature of 25 °C and with a ratio between YCC and headspace of 118.18 million yeast cells per ml of headspace volume in a closed fermenter.

### 6     Summary

The current research work had the objective of developing an analysis method for determining yeast vitality based on gas pressure build-up, assessing the metabolic reactions of brewer's yeast. This should provide a tool for evaluating the fermentative capacity of a yeast batch more rapidly and relatively easily, e.g. prior to pitching. In addition, a direct relationship was found between the official methods for determining yeast vitality according to Hlaváček and the intracellular pH value of the yeast (ICP) and the analysis method developed in this research work. Compared to other methods, the analysis proposed has a higher sensitivity

in differentiating between mediocre and bad yeast vitality, based on gas formation. For the first time, precise observation of the lag-phase is possible by graphically representing pressure build-up in real time.

According to the results yeast batches having good vitality should, be able to build up a calculated pressure of 1 bar in a time frame shorter than (140 min/σ = 7%) when running in accordance with the test conditions specified, i.e. bottom-fermenting lager beer wort with about 12 °P, the TUM 34/70 yeast strain, 65 million YC/ml, 25 °C and 118.18 million YC/ml headspace.

The induction phase required for carbon dioxide evolution (referred in this contribution as $CO_2$ lag-phase) closely correlates with the lag-phase of the yeast propagation cycle. The lag-phase in operation should thereby be as short as possible, in order to avoid the growth of bacteria and in order to increase the efficiency and capacity of the fermentation process in the brewery. Hence, this parameter should be evaluated and monitored regular.

## 7    Conclusion and outlook

In conclusion, the method presented here provides a means of assessing the fermentative capacity of a yeast batch more rapidly and easily, e.g. prior to pitching, compared to conventional methods. Based on empirical values, sensitivity and precision of the method presented here can be regarded as being very high as small modifications brought about changes in fermentation behavior.

Further research work was carried out in order to determine whether stressors should be included on purpose in the analysis in order to raise the sensitivity of the method. The results of that investigation will be published immediately after the present publication. Further investigations will be aimed principally at identifying the technology for optimizing propagation and fermentation in a simpler and more precise manner in breweries.

The analysis method presented here is already a useful tool for brewers. Only when the physiological condition of yeast is diagnosed at an early stage is it possible to adopt measures required for safeguarding and maintaining quality, productivity and thus, ultimately, the image of the product.

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