The sensory characteristics of beer are influenced by various aromatic substances, including those produced by yeast in the fermentation and maturation process and those produced as a result of further biochemical and chemical reactions, and complex interactions (Titica et al. 2000).

During wort fermentation, inter alia, aldehydes, which have a major effect on the sensory characteristics of beer and its taste stability, are being produced. In general, it may be assumed that aldehydes, as well as other carbonylic compounds (vicinal diketones), have a negative impact on the characteristics of the beverage because of the low sensory threshold and high reactivity (Vanderhaegen et al. 2007).

A relatively high amount of acetaldehyde is produced during the first three days of this process. Small quantities of this component may have a beneficial effect on taste and aroma characteristics, and also, in an indirect way, through the formation of acetics. In higher concentrations, usually above 3–4 mg/l, it contributes to a typical, grassy, a bit apple taste and aroma similar to that contributed to beverages by alcohol and hexaldehyde (Eßlinger 2009). During taste evaluation this defect is commonly defined as characteristic of green, immature beer with a cellar note (Pickerell et al. 1991).

Nowadays, fermentation tanks (cylindrical-conical tanks – CKT) are used in beer production in most breweries. Those are modern tanks of different sizes adjusted to the brewery output, which facilitate the production process, and provide sterile conditions for beer manufacturing.

The wort fermentation process and beer maturation have a huge impact on sensorial characteristics. During those phases, the final sensory profile of the beverage is being shaped. Apart from the yeast strain, relevant technological parameters are of great importance. The main factors affecting the fermentation process and beer maturation are temperature, yeast pitching rate, initial level of wort aeration, and the way of filling fermentation tanks.

Earlier work of Kucharczyk and Tuszyński (2015) presented that the reductions in the concentration of acetaldehyde were obtained by increasing the
inoculum dose from $5 \times 10^6$ to $9 \times 10^6$ cells/ml of wort. In turn, sensory properties of beers produced with larger initial amounts of yeast were evaluated more poorly.

The other factor that influences the fermentation and intensifies the biochemical processes is the fermentation tank filling time.

Studies already carried out conclude that the essential point is to provide an additional dose of oxygen in the next part of aerated wort which is being filled. This is the moment in which yeast is undergoing the phase of budding and propagation. As an effect, better quality yeast, with a large volume of young cells with high vitality that reduce aldehyde formation, is obtained.

Yeast cells, thanks to maintaining the appropriate content of glycogen and trehalose, are then more resistant while in new conditions (pitching the wort) to different kinds of stress. In consequence, nutrient uptake is optimal and metabolite exchange is selective. A relatively short fermentation tank filling time may lead to delayed fermentation, slow primary fermentation, and to a higher volume of undesired components of the green beer bouquet, such as acetaldehyde and diacetyl.

Younis and Stewart (1999) emphasised that the most stimulating moment, suitable for aeration, is the secondary yeast budding phase. This is the time when the highest amount of oxygen is needed for biosynthesis of unsaturated fatty acids. During budding the content of lipids in cells is reduced drastically.

Additional biomass oxygenation is conductive to sterol synthesis in the cell wall (Maemura et al. 1998). Sufficient oxygen availability to yeast, especially at the beginning of fermentation, is related to lipid synthesis, which is inhibited as long as fatty acids, and in consequence esters, are created (Lee 1999).

Other experiments performed on a laboratory scale have also confirmed the beneficial effect of appropriate biomass and wort oxygenation on, among others, reducing the content of acetaldehyde (Jones et al. 2007).

Furthermore, Lodolo and Cantrell (2005) showed that the optimal filling time ensures that the fermentation process runs correctly and the proper harmony of volatile components in beer is obtained.

To sum up, it can be stated that a very important phase of the process, which has an effect on the fermentation and the final content of acetaldehyde in the finished product, is the wort fermentation. This phase depends on, inter alia, properly adjusted fermentation tank filling time. Filling the fermentation tank gradually with well aerated wort not only leads to an increase in fermentation speed, but also it influences the empirical formula, beer sensorial characteristics, and viability of yeast cells which can be used again in the next production cycle.

**MATERIAL AND METHODS**

*Execution of experiments.* The focus of this study was a parallel process of beer production in three fermentation tanks with a capacity of 3800 hl, from which samples were taken during 18 days of the whole production cycle. Each tank was filled with three batches of wort, 1030 hl each. High-gravity worts – HG (15.5′Bgl) were prepared using the same malt part and with identical technological conditions. Sample collection was started after filling the CKT and was continued during the next days at the same time every day. *Saccharomyces carlsbergensis* (W34/70) yeasts were used in the fermentation process – they were collected after the 2nd fermentation (3rd passage) in a quantity of 7 ml cells per 1 ml wort, which were stored in the same yeast tank. The worts were aerated with sterile air in an amount of 10 mg/l. Each CKT was filled in three different time periods: A – 4.5 h, B – 9 h, and C – 13.5 h, according to the agreed tank filling interval introduced after the first beer batch. Fermentation process and beer maturation in all fermentation tanks were performed in the same technological conditions.

*Analytical procedures.* Apparent extract measurements were performed using an automatic wort and beer analyser (Beer Analyser DMA 4500 M; Anton Paar, Graz, Austria). Density at 20°C and the specific weight was marked using an oscillating density meter. The Tabarié formula was the basis for alcolyser beer calculations (Miedaner 2002).

Qualitative and quantitative analyses of volatile components (the identification was done on the basis of retention time) were performed using a GC 8000 gas chromatograph (Fisons Instruments, Ipswich, UK) fitted with GC-FID flame ionisation detector for detection of acetaldehyde, ethyl acetate, sum of higher alcohols and GC-ECD detector for detection of diacetyl, 2,3-pentanedione.

The DB-WAX capillary column (dimensions: 60 m long, 0.53 mm internal diameter, and 1 µm thick) packed with polar polyethylene glycol was used for the separation. The mixture of 3-panthenol and n-butanol was used as an internal standard for the
determination of acetaldehyde, ethyl acetate, and sum of higher alcohols.

The CP-Sil8CB capillary column (60 m long, 0.25 mm internal diameter, and 1 µm thick) packed with a nonpolar material (5% phenyl and 95% dimethylpolysiloxane) was used for the determination of diacetyl and 2,3-pentanedione.

The total amount of yeast cells and their viability during the fermentation and maturation of beer were determined with a NucleoCounter YC-100 (Chemometec, Lillerød, Denmark). This system identifies and counts single cells by propidium iodide as a DNA stain.

**Sensory analysis.** Sensory evaluation of bottled beer used a comparison test, with the test sample compared to the reference beer profile. The beer was tested in special black glasses. Profile tests involved the evaluation of beer attributes, including aroma esters, hops, bitterness, sulphur compounds, sweetness, acidity, fullness, balance, and flavour. The beer was evaluated (nine-member brewery trained panel) according to a scale from 2.7 to 4.3 points (very good: 2.7–3.0; good: 3.0–3.3; neither good nor poor: 3.3–3.7; poor: 3.7–4.0; very poor: 4.0–4.3).

**Statistical analysis.** Results presented in this work were the average of three independent experiments with the bars representing the standard deviation. Data were analysed by analysis of variance (ANOVA) to test the significance of fermentation tank filling time in relation to the concentration of acetaldehyde in beer and other parameters. Significant differences between the means were verified by Duncan’s test ($P < 0.05$) with the use of STATISTICA Version 10 (StatSoft Polska, Kraków, Poland).

**RESULTS AND DISCUSSION**

The influence of the filling time of fermentation tanks on the fermentation process is illustrated in Figure 1. The processes of beer fermentation and maturation in the fermenters were conducted under the same process conditions: primary fermentation at 10°C, then warm and cold maturation at 13°C and –0.5°C, respectively. Similar changes in the apparent extract content in fermentation tanks filled with an interval kept between the first and next beer batches (CKT filling time 9 and 13.5 h) are characteristic. This is also confirmed by the apparent extract drop after 120 h from the process initiation (Figure 2). During the analysed period about 70% of the studied wort extract decreased and it was gradually replenished in the fermentation tank. The fermentation tank filled without intervals, during 4.5 h, was characterised by low apparent attenuation (about 53%). Consequently, the fermentation process in tanks filled with the use of the interval system was shorter (6 days) than parallel attempts of filling with the use of the non-interval method (8 days).

Constant refilling of the tank with next beer batches during those 4.5 h resulted in slower process kinetics – the average apparent extract decrease was 1.66°Blg/day, whereas filling the tanks with intervals during 9 and 13.5 h resulted in faster daily extract attenuation to around 2.20°Blg – about 35% of fermentation acceleration in relation to filling the tanks continuously with next worts. Based on those experiments it can be stated that the optimal filling time is 9 hours.

The higher extract attenuation speed results from greater biomass growth, which is the effect of introducing additional nutrients and oxygen with every next wort batch. The important factor here is the time after which the next part of aerated wort needs to be added. This parameter should be modified depending on the concentration and volume of the wort.

Longer intervals between the first and next batches (raised from 9 h to 13.5 h) did not affect the fermentation speed.
Our study showed that fast wort fermentation and an appropriate filling time have a beneficial effect on the empirical formula (lower acetaldehyde content) and in consequence on the sensorial characteristics of beer.
As Table 1 shows, different fermenter filling time used in the experiments had no significant effect on the studied volatile components such as diacetyl and 2,3-pentanedione. The concentration of diacetyl was around 20–24 µg/l, which indicates a very low level (100 µg/l is the human threshold). In turn, the time of filling CKT has an impact on the concentration of ethyl acetate and on the sum of higher alcohols. Previous works carried out on a different scale and with different technological conditions showed that the prolonged time of filling has an influence on yeast oxygenation and also on an increase in the amount of esters (Verbelen et al. 2009).

Jones et al. (2007) demonstrated that ethyl acetate increased with the second portion of fresh oxygen delivered after 12 hours.

Experiments have shown that different fermenter filling time used in the experiments had no significant effect on the sensory quality of the final product. Although all beers were evaluated as “good” on a scale of 3.3 to 3.25 (Figure 4), on the basis of sensory evaluation it can be concluded that the longest time of fermenter filling (13.5 h) favoured the sensory evaluation of the product. Better taste may be mainly related to lower acetaldehyde content and higher concentration of esters.

The research carried out implies that it is crucial to provide additional oxygen with each portion of well aerated wort added. It is the time when the yeast undergoes the second phase of budding. During the interval in tank filling, the yeast propagates intensively and provides more young cells, which are a guarantee of the intensity of metabolic changes, shortening the fermentation time and lowering the acetaldehyde content. This is also the time of the greatest demand for oxygen used in unsaturated fatty acid biosynthesis. This is so because during budding the lipid content in the yeast cell drastically decreases.

### CONCLUSION

In industrial conditions, using multiple fermentation tank refilling with fresh aerated wort enables higher production effectiveness in breweries because of the accelerated beer wort fermentation.

Prolonged wort aeration results in faster fermentation and has a positive impact on a decrease in the content of acetaldehyde.

### References


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Table 1. Impact of different fermenter filling time on the final concentrations of acetaldehyde

<table>
<thead>
<tr>
<th>Flavour compound</th>
<th>Time filling fermenters (h)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>0.024</td>
<td>0.022</td>
</tr>
<tr>
<td>2,3-Pentadion</td>
<td>0.017</td>
<td>0.014</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>18.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sum higher alcohols</td>
<td>92.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*display the significance at 5%; ns – not significant; <sup>a</sup>according to Duncan’s test means within columns followed by the same letter are not significantly different

Figure 4. Quality of beer depending on different fermenter filling times

Values are means ± SD (n = 3), the letters indicate homogeneous groups


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