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Effect of Wort Filling Time on Fermentation, Maturation and Acetaldehyde Content in Beer

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Abstract

Kucharczyk K., Tuszyński T. (2016): **Effect of wort filling time on fermentation, maturation and acetaldehyde content in beer**. Czech J. Food Sci., 34: 265–270.

The effect of wort filling time on the process of fermentation, maturation, and acetaldehyde content in beer was determined. The experiments were performed on an industrial scale, the fermentation and maturation took place in fermentation tanks. Three tanks were filled using three different intervals. Worts were aerated with sterile air and yeast was added after the second fermentation (third passage). During fermentation and maturation, changes in the content of the apparent extract and the amount of acetaldehyde were investigated. Experiments have shown that different filling times have a significant impact on the course of fermentation and the amount of acetaldehyde. With the increase of wort filling time, fermentation speed improved and acetaldehyde content decreased.

Keywords: fermentation tank; industrial scale; volatile components; apparent extract; quality of beer

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 acetals. 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

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inoculum dose from 5×10^6 to 9×10^6 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°Blg) 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

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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

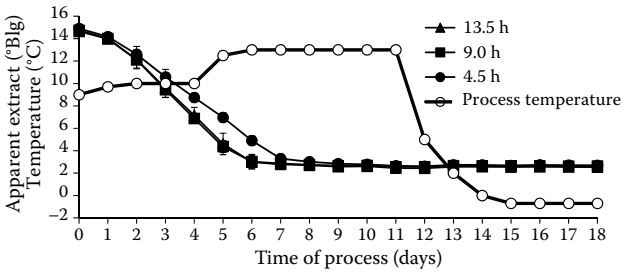

Figure 1. Apparent extract drop at three different fermenter filling times (h)

Values are means ± SD (n = 3)

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.

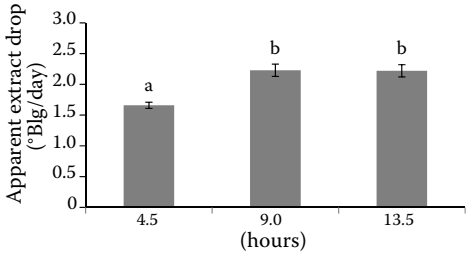

Figure 2. Average daily apparent extract drop at different fermenter filling times (hours)

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

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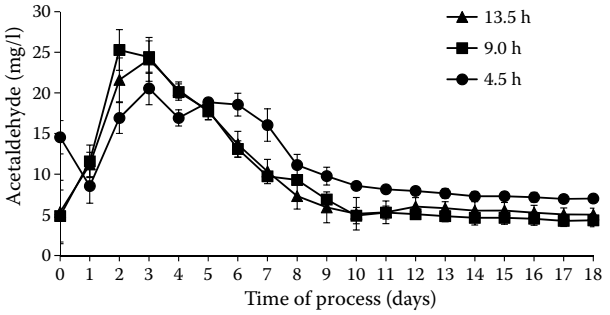

Figure 3. Formation of acetaldehyde content according to different fermenter filling times (h)

Values are means ± SD (n = 3)

Yokoyama and Ingledew (1997) showed that filling the already fermenting wort with fresh portion after 6 h allows obtaining a much better yeast biomass condition and has a significant impact on the beer empirical formula.

Jones *et al*. (2007) obtained similar results by replacing wort filling with additional aeration after 12 h from the bioreactor filling, which gave them fermentation shorter by 30%.

Other studies carried out on a micro and macro scale also show that the longer wort aeration process relates with yeast viability, also during fermentation and maturation, which significantly contributes to fermentation intensification (Verbelen 2009).

Changes in the amount of acetaldehyde during fermentation and maturation depending on the tank filling time are illustrated in Figure 3. Before analysing the acetaldehyde content in the wort during fermentation and maturation, 0.5 mg of this compound was marked in 1 l of cooled wort before the yeast was added. Longer intervals between fillings contributed to an explicit decrease of acetaldehyde content in the initial test after the fermentation tank had been filled. This is related to the faster yeast cell replication already occurring while filling the tank. When filling the tank without intervals between each beer batch (filling time 4.5 h), the content of acetaldehyde was around 15 mg/l, while in tanks filled with intervals (filling time 9 and 13.5 h) the content of this compound was lower – around 5 mg wort/dm^3 (initial stage of fermentation process). When comparing the acetaldehyde content, it can be stated that during 4.5 h (difference in the filling time between 9 h and 4.5 h) a significant decrease of ethanal occurred.

On the first day of the process, the content of acetaldehyde in all CKTs equalised and ranged from 8 to 12 mg/l. During the next day, acetaldehyde concentration increased rapidly and showed a difference in the content between the tanks with filling time equal or over 9 h and the tank filled in a shorter time (4.5 h). It can be stated that the phase in which acetaldehyde content increased lasted almost 3 days and only in the tanks filled in 9 h it was usually shorter (2 days). After that time, the ethanal reduction phase started in the CKTs filled with an interval and lasted until the yeast harvest, which is the 10th day.

On the other hand, the reduction of this compound in the tanks filled without interval occurred within 4.5 h in a different way. After a short acetaldehyde reduction, on the 4th day the process was untypically inhibited and then the compound content started to rise again. On the 5th day ethanal concentration in all tanks was comparable. Since the 6th day the reduction proceeded with similar intensity like in the other two tanks and remained like that until the 11th production day – yeast cropping.

Varied kinetics of those changes is undoubtedly connected with the yeast fermentation activity identified by changes in the content of the extract (Figure 1).

The statistical analysis shows that there is no significant difference in aldehyde concentration between 9 and 13.5 h filling time. In both cases the average content of this compound is around 8.6 mg/l. A comparable concentration of acetaldehyde may result from similar yeast biomass growth and kinetics of the fermentation process in discussed CKTs.

Fermented worts in tanks filled using the non-interval system (4.5 h) were characterised by a 4 mg higher content of acetaldehyde, which was reduced to 2 mg in the final stage of maturation.

These results clearly illustrate the beneficial effects of filling the fermentation tanks after a several-hour break. The statistical analysis in Table 1 demonstrates that the average content of acetaldehyde differed significantly ($P < 0.05$) depending on the filling model. The extension of the tank filling time (introducing a break in filling) had a beneficial impact on acetaldehyde content in beer, which was decreased by 20%.

Studies carried out by other researchers (Lodolo & Cantrell 2005; Verbelen 2009) prove that higher aeration, which occurs when the wort is filled several times, warrants better beer quality. Cheraiti *et al*. (2010) demonstrated that prolonging the logarithmic yeast growth phase had a significant impact on acetaldehyde content.

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.

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

Flavour compound (mg/l)	Time filling fermenters (h)[y]			Significance
	4.5	9.0	13.5	
Acetaldehyde	6.8[c]	4.3[a]	5.6[b]	*
Diacetyl	0.024	0.022	0.019	ns
2,3-Pentadion	0.017	0.014	0.013	ns
Ethyl acetate	18.8[a]	19.4[ab]	19.7[b]	*
Sum higher alcohols	92.7[a]	107.8[b]	110.8[b]	*

*display the significance at 5%; ns – not significant; [y]according to Duncan's test means within columns followed by the same letter are not significantly different

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 had a low but 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.

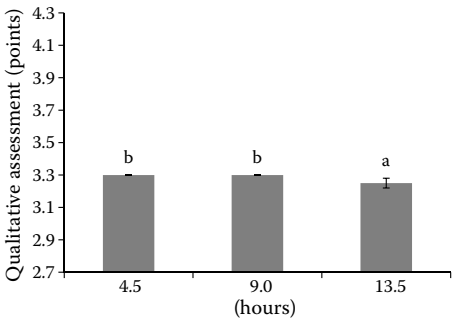

Figure 4. Quality of beer depending on different fermenter filling times

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

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.

CONCLUSSION

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.

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