Effect of Yeast Harvest Moment on a Brewing Process in Beer Produced on an Industrial Scale

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Abstract

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The aim of this study was to determine the effect of yeast harvest timing on the process performance, total yeast count and the content of volatile components in beer. The experiments were performed on an industrial scale with fermentation and maturation conducted in three fermentation tanks with a capacity of 3800 hl (cylindro-conical tanks – CCT). All processes were carried out using the same technological conditions. The worts were aerated with sterile air and yeast after the second fermentation (third generation) was added. The duration of the maturation phase and the processes of the yeast harvest were conducted at different times (1st, 4th and 6th day) after finishing the primary fermentation process. During fermentation and maturation, changes in the contents of the extract, yeast, and volatile components were investigated. These experiments showed that the use of different times during yeast harvest had a significant impact on the course of fermentation and maturation and impact on the total yeast count during the maturation process and on the amount of volatile components in beer. With a delay in the start of yeast cropping, the content of acetaldehyde and vicinal diketones decreased and the content of esters increased. The timing of the yeast crop significantly influenced the final beer quality.

Keywords: beer; cylindro-conical tank; volatile components; yeast harvest; wort

Beer volatile component quality and quantity depend mainly on the chemical composition of the wort and its aeration level; pitching yeast strain and dose, and their vitality, fermentation and maturation process temperatures; fermenting tank filling method; and other traits (Uchida & Ono 1999; Jones *et al.* 2007; Kucharczyk & Tuszyński 2015, 2016).

The various yeast management processes that include the mechanical or physical treatment of yeast are collectively called 'Yeast Handling'. The modern yeast handling circuits are designed for the movement of yeast from one vessel (fermenter) to the next. During the yeast handling cycle, the brewing yeast (slurry) is recovered (cropped) with the use of cropping pumps from the cone of the cylindro-conical fermentation vessel (CCT) after the fermentation process (Lodolo *et al.* 2008).

Yeast flocculation characteristics dictate the fermenter design. CCTs are ideally suited to lager strains (bottom yeasts) because the cells clump together, resulting in flocs that sediment from the medium to settle in the bottom of CCT cones. This strain-dependent phenomenon is termed flocculation (Speers *et al.* 1992; Verstrepen *et al.* 2003).

In the fermentation process, the timing of flocculation is important. Flocculation should not take place too early, before the wort is completely attenuated, because premature flocculation causes sluggish or stuck fermentation and produces beers with a high residual fermentable sugar content, resulting in unsatisfactory flavour characteristics. Instead, strong and virtually complete flocculation at the end of the fermentation is desired, providing a cheap, effective and environmentally friendly way to remove nearly,

but not quite, all yeast cells from the green beer (Virve & Londesborough 2011).

The specific manner of harvesting, yeast storage and treatment can also have a specific effect on flocculation, especially when the yeast is collected from the bottom of the fermenter, as is the case in today's breweries. The yeast sediment from which the cropping is made at the end of fermentation is not homogenous: older and/or more flocculent cells will sediment earlier, resulting in an enrichment of these cells near the bottom and middle part of the cone. Similarly, young and/or non-flocculent and weakly flocculent cells will be found mostly in the top layers of the yeast sediment (Verstrepen *et al.* 2003).

At the end of fermentation, a portion of the yeast is removed ('cropped') from the fermentation vessel for serial repitching. Typically, this is the centre-top portion of the yeast crop, theoretically consisting of middle-aged and virgin cells. Harvesting yeast may therefore select for a population with an imbalance of young or aged individuals, depending on the cropping mechanism employed (Powell *et al.* 2003). The timing of the yeast harvest from the cylindro-conical tank leads not only to a proper maturation process but also influences the beer sensorial characteristics and the viability of the yeast cells, which can then be used again in the next production cycle.

To sum up, it can be stated that yeast cropping is a very important phase of the process, with an effect on the fermentation performance and the final content of volatile components in the finished product. This operation depends on properly selecting the time to harvest the yeast.

The goal of these experiments was to determine the effect of yeast harvest timing on the processes of fermentation and maturation, concentrations of the volatile components of beer and viability of yeast biomass on a technical scale. It should be noted that both the aim and the novelty of this study are the comparison of the results obtained at the laboratory and industrial scale and the improvement in knowledge concerning this issue.

MATERIAL AND METHODS

Execution of experiments. The focus of this study was a parallel process of beer production in three fermentation tanks (CCT) with a capacity of 3 800 hl, from which samples were taken during the 18 days of the whole production cycle. Each tank was filled with three batches of wort, 1030 hl each. HGB (high gravity 15.5°P) worts were prepared from the same batch of malt under identical technological conditions. A pilsner type malt from two malt houses was used in the experiments. The process of infusion mashing-in took place at 60–76°C. Afterwards, the mash was transferred to a lauter tun.

Sample collection was started after filling the CCT and was continued at the same time every day. *Saccharomyces carlsbergensis* (W34/70) yeast were used in the fermentation process. They were collected after the second fermentation (third passage) in a quantity of 7 mil cells per 1 ml wort, which were stored in the same yeast tank. The worts were aerated (compressed, sterile air under 4 bar pressure was pushed through the wort line, which had a pressure of 2.5 bars) during transfer to each CKT, with this same amount of 10 mg O_2/l wort. The duration of the maturation phase from three independent tanks (A, B, and C) and the operation of yeast harvesting were conducted on the 1st, 4th, and 6th day after completing the fermentation process. The fermentation processes and beer maturation in those fermentation tanks were carried out using the same technological conditions: primary fermentation at 10°C, then warm and cold maturation at −1°C.

Analytical procedures. Apparent extract measurements were performed using an automatic wort and beer analyser (Beer Analyzer DMA 4500+; Anton Paar, Austria), density at 20°C, and the specific weight was marked using an oscillating densitometer. Tabarié's formula was the basis for the 'Alcolyzer' beer calculations (Miedaner 2002).

Qualitative and quantitative analysis of volatile components (the identification was done on the basis of retention time) was performed using a gas chromatograph GC 8000 (Fisons Instruments, UK) fitted with a flame ionisation detector GC-FID for determination of acetaldehyde and ethyl acetate, and of the sum of higher alcohols and detector GC-ECD for detection diacetyl, 2,3-pentanedione. A capillary column DB-WAX (dimension 60 m long, 0.53 mm i.d. and 1 μm thick) packed with polar polyethylene glycol was used for the separation. A mixture of 3-panthenol and *n*-butanol was used as an internal standard for the determination of the acetaldehyde, ethyl acetate and sum of higher alcohols. A capillary column CP-Sil8CB (60 m long, 0.25 mm internal diameter and 1 μm thick) packed with a nonpolar material (5% phenyl 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 the use of the NucleoCounter YC-100 (Chemometec, Denmark). This system identifies and counts single cells with DNA stained by propidium iodide.

Sensory analysis. Sensory evaluation of bottling 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 the attributes of the beer, including aroma esters, hops, bitterness, sulphur compounds, sweetness, acidity, fullness, balance and flavour. The beer was evaluated by a panel of nine trained brewers according to a scale from 2.7 to 4.3 points (very good: 2.7–3; good: 3–3.3; neither good nor poor: 3.3–3.7; poor: 3.7–4; very poor: 4–4.3).

Statistical analysis. The results presented in this work are the average of three independent experiments with the bars representing the standard deviation. The data were analysed by one-way analysis of variance (ANOVA) to test the significance of the different fermentation temperatures on the concentrations of the volatile components in the beer and other parameters. Significant differences between the means were verified by Duncan's test ($P < 0.05$) with the use of Statistica v.10 (StatSoft Polska, Poland).

RESULTS AND DISCUSSION

The timing of pumping out the yeast from the fermentation vessel after the finished process of fermentation is one of the most important technological innovations in beer production. The proper determination of this moment influences the desired content of the volatile components in beer and the physiological condition of the collected yeast, which is used for subsequent fermentations.

In Figure 1, we show the timing of the collection of the yeast from the three examined fermenting tanks. The collection of the yeast took place on 1, 4 and 6 days after completion of the fermentation process. Because the fermentation process and the process of maturation were carried out under the same conditions (time, temperature and pressure), the graph shows identical changes of apparent extracts in the examined fermenting tanks. The average loss of the extract amounted moderately to approximately 1.80°Blg per day. A decrease in the amount of the extract to 3.4°Blg meant the fermentation process had finished and indicated the beginning of the maturation process on the seventh day of the process.

In Figure 2, we present the changes in the number of yeast cells during fermentation and maturation depending on the timing of the yeast collection from the fermenting tanks. The course of the graph shows the constant dependency of the increase in biomass during the process of fermentation in the examined trials. Until the fourth day of the process, there was a more than five times increase in the number of cells (up to 37 million cells per 1 ml). The changes in the number of yeast cells in the beer were tracked after finishing the fermentation and during the recovery of the yeast in the subsequent days during the maturation process.

In the case of the fermenting tank from which the yeast were taken (on the seventh day), during the next few days of the technological process, the lowest number of yeast cells was found, at approximately 5 million cells per 1 ml of beer. The next highest number of yeast cells suspended in beer (approximately 10 million cells per 1 ml) was found in the case of the beer batch from which the yeast was taken much later (on the twelfth day of the process).

From our analysis, it appears that the best moment of yeast collection after the finished fermentation is right at the beginning of the maturation process. In
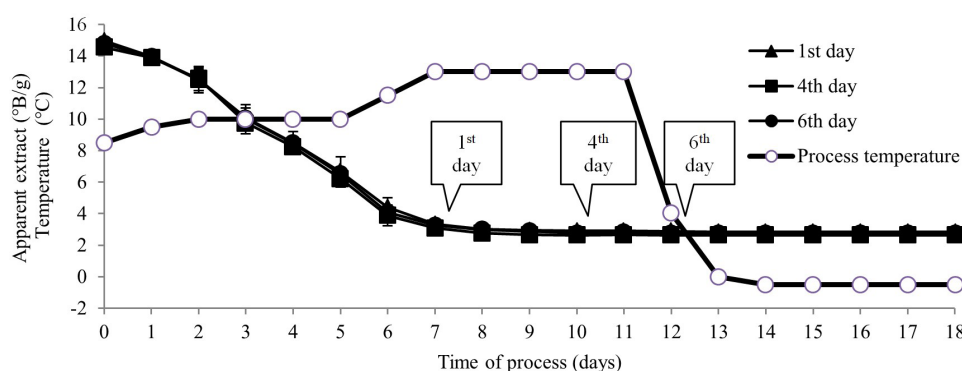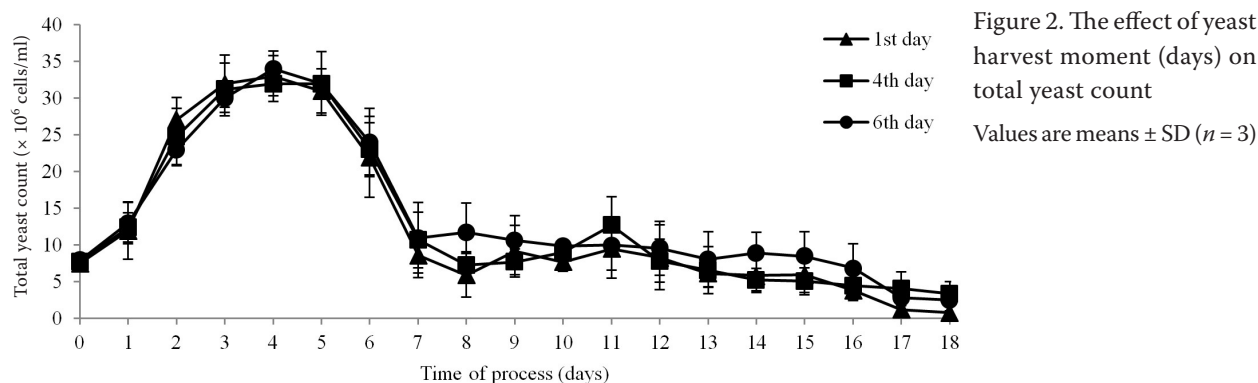

Figure 1. Apparent extract drop at three different yeast harvest moment (days)

Values are means ± SD ($n = 3$)

Figure 2. The effect of yeast harvest moment (days) on total yeast count

Values are means ± SD (*n* = 3)

the gathered (collected) biomass, the proportion of the young and old yeast cells is not disturbed.
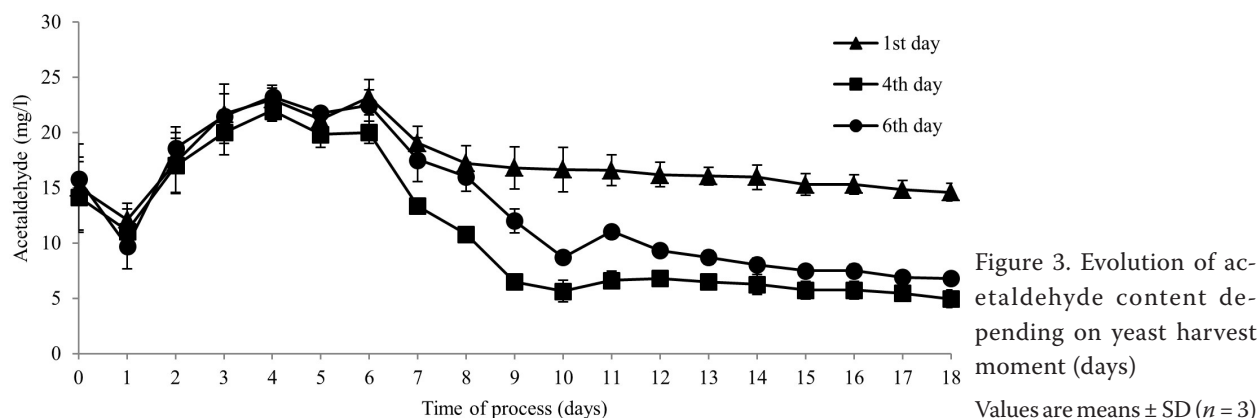
Estimation of the influence of timing of the yeast slurry collection on the final products characteristics was the purpose of a study by Powell *et al.* (2000). The authors showed the need to establish an optimum moment of yeast collection after finishing the fermentation process. The authors found that collecting the yeast too early causes an increase in the participation of older yeast cells, which has an influence on extension of the lag phase and delays the fermenting process. Then, the increased proportion of young cells obtained as a result of the delayed collection causes a slowed initial growth of the biomass of the yeast and simultaneously extends the fermentation process. Numerous authors quoted in a review publication (Virve & Londesborough 2011) also supported the hypothesis that the timing of yeast collection from the cone of the fermenting tank has a significant influence, among others, on the flocculation of the yeast slurry.

After collection of the biomass from the cone part of the tank, the remaining suspended yeast cells in the maturating beer decreased until finishing the process on the eighteenth day, attaining a concentration from 1 to 4 million cells per ml, based on the batch

of beer from which the yeast was taken on the first and fourth day of maturation. One of the advantages of the earlier collection of yeast from the fermenting vessel is its lower concentration in mature beer, which undoubtedly has a beneficial influence on the filtrating properties of beer.

The changes in the amount of acetaldehyde at the time of fermentation and maturation was also dependent on the time of collection of yeast from the fermenting tanks, as shown in Figure 3. Before starting the process, in the cooled pitching wort (without the addition of yeast) the acetaldehyde levels were relatively low and amounted to approximately 0.5 mg/l.

On the first day of the process, after 12 h of fermentation, in all examined fermenting tanks, the amount of acetaldehyde amounted to approximately 15 mg/dm^3, and on the second day (after 30 h of fermentation) decreased to around 10 mg/dm^3. This is probably correlated with fast yeast multiplication during filling of the fermenting tanks. On the last (sixth) day of fermentation, the amount of acetaldehyde in the examined batches of beer oscillated approximately 20 mg/l and then began to decrease. Diametrical changes in acetaldehyde concentrations appeared during the collection of yeast from the fermenting tanks.

Figure 3. Evolution of acetaldehyde content depending on yeast harvest moment (days)

Values are means ± SD (*n* = 3)

An earlier collection of yeast (the first day of maturation) contributed to a clear decrease in the degree of reduction of the acetaldehyde content. In this period, the acetaldehyde concentration was approximately 18 mg/l. In the next few days of maturation, the amount of acetaldehyde decreased and finally, on the eighteenth day of the process, the acetaldehyde concentration developed to a level of 14 mg/l. In the case of the fermenting tanks in which at this stage the yeast was still present, the amount of acetaldehyde was decreasing.

From the second fermenting tank, the yeast was removed on the fourth day of maturation. At the moment of collecting the yeast, the amount of acetaldehyde was approximately 5 mg/l. After this, there were no further changes until the last analysed day in the process. In the case of pumping out the yeast on the sixth day of the maturation process from the third fermenting tank, the development of acetaldehyde concentration was very similar to the course of the process of the production of the beer from which the yeast was separated 2 days earlier. There was no statistically significant difference in the amount of acetaldehyde between the collection on the fourth and sixth day of the maturation process.

In the context of this research carried out under industrial conditions, we need to state that an earlier separation of yeast leads to an excessive amount of acetaldehyde in the beer, as much as three times higher. Our experiments showed that the optimum time of yeast collection is the fourth day after the completion of wort fermentation of an initial extract at 15.5°Blg.

Previous research (ERTEN *et al.* 2007) proved that the collection of yeast too early, despite a high initial concentration of breeding yeast cells, led to the occurrence of an increased concentration of acetaldehyde due to its incomplete reduction. Similar results were attained by VERBELEN *et al.* (2009), who showed a close dependency between shortening of fermentation time and increases in the amount of acetaldehyde. However, the unfavourable change of the amount of acetaldehyde, in the case of shortening of the fermentation time, as a result of earlier separation of the yeast, may also take place due to a faster flocculation of the yeast (YU-LAI JIN & SPEERS 2000).

The statistical analysis presented in Table 1 shows that the timing of the collection of yeast after finishing the fermentation also has a significant influence on the amount of the other examined volatile components of beer, mainly diacetyl, 2,3-pentanedione, esters and DMS. As a result of the earlier collection of yeast on the first day of maturation, the amount of diacetyl reached a level of 27 μg in one litre of beer, whereas a later separation of biomass resulted in a lower level, approximately 30% on average. In the case of 2,3–pentanedione, a later yeast collection also caused decrease of this component by approximately 50%.

In the previous studies, VERBELEN *et al.* (2009) and NGUYEN and VIET MAN (2012) proved that shortening the fermentation process through an earlier yeast collection caused a higher level of undesirable volatile components, mainly diacetyl and 2,3-pentanedione. The lack of chemical oxidative decarboxylation and the consecutive biochemical reduction by the residual yeast during maturation influenced the content of undesirable flavours, such as those from diacetyl. The research carried out here also showed that a longer period of storage of yeast in fermenting tanks has an influence, increasing the amount of esters, ethyl acetate and isoamyl acetate by approximately 10%.

Table 1. Impact of moments of yeast crop from the cylindro-conical tanks (CCTs) on the final concentrations of volatile components

Flavour compound (mg/l)	Moment of yeast harvest (day)[y]			Significance
	1st	4th	6th	
Acetaldehyde	14.83[c]	5.20[a]	6.77[b]	*
Diacetyl	0.03[b]	0.02[a]	0.02[a]	*
2,3-Pentanedione	0.03[b]	0.02[a]	0.02[a]	*
Ethyl acetate	16.57[a]	18.86[b]	18.43[b]	*
Isoamyl acetate	1.61[a]	1.77[b]	1.75[b]	*
DMS	0.06[b]	0.05[a]	0.07[c]	*
Sum higher alcohols	96.97	95.86	97.87	ns

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

However, the experiments carried out by VERBELEN *et al.* (2009) did not confirm a smaller amount of ethyl acetate due to a more intensive fermentation and a shorter time of yeast left in the fermenting tanks. Similar results were reported by ERTEN *et al.* (2007), who confirmed that faster cropping of yeast, as a result of a higher speed of fermentation, had no significant impact on the content of ethyl acetate. EDELEN *et al.* (1996) also showed a decrease in the amount of esters in response to accelerated collection of the yeast.

For the case of isoamyl acetate content, with increasing contact time of the yeast with beer in the CCT, the amount of isoamyl acetate in beer significantly increased. As shown in the study by ERTEN *et al.* (2007), a shorter exposure to the yeast in the fermenting tanks reduced the isoamyl acetale content in lager beer. However, a later work of VERBELEN *et al.* (2009), who reported that faster yeast cropping increased the concentration of isoamyl acetale, seems to be in conflict with the results of our studies.

These discrepancies may be caused by differences in the yeast strain physiology and by the scale of the fermentation vessels employed in those studies and methods used for inoculation of the yeast (strain and pitching rate).

There are no previous trials that researched the effect of different timings of yeast cropping on alcohol concentration. Experiments conducted by us clearly indicate (Table 1) that the yeast presence time in the CCT had no significant influence on the sum of higher alcohols in beer. Higher alcohols are excreted by yeast into beer until fermentation is completed. Generally, all technological parameters that affect the intensive fermentation speed causes a higher yield of the synthesis of fusel alcohols. Therefore, despite earlier yeast harvesting as a result of a shorter fermentation, the beer contains more higher alcohols. The results presented by JONES *et al.* (2007) confirm such a relationship. An increased content of fusel alcohols is the result of a higher yeast pitching rate. In yet another study (LIMA *et al.* 2011), it was confirmed that a higher pitching rate resulted in increasing concentrations of propanol. In the study carried out by LANDAUD *et al.* (2001), it was stated that a higher alcohol content was due to a higher temperature of fermentation. In our earlier study (KUCHARCZYK & TUSZYŃSKI 2016), a prolonged contact time of the yeast with the beer in the fermenting tanks as a result of an extended filling time of the CCTs (from 4.5 to 13.5 h) resulted in a 20% increase in the concentration of higher alcohols.
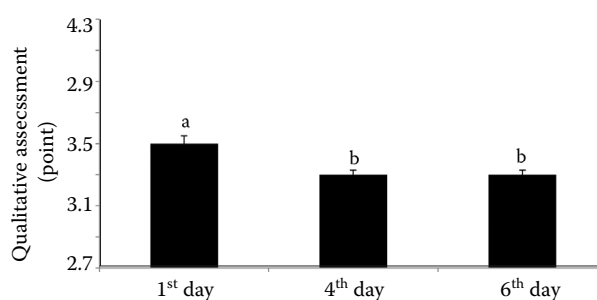

Figure 4. Quality of beer depending on yeast harvest moment (days)

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

The experiments carried out showed that the timing of the separation of the yeast from the beer in the examined fermenting tanks had a significant influence on the quality of the final product (Figure 4). An earlier collection of yeast (on the first day of maturation) negatively influenced the sensory properties of the beer. The beer from this fermenting tank was given a mark of 3,5, i.e., within the range 'neither good nor poor'. The beers produced from later yeast collections were definitely marked as better by the tasting panel. These beers were marked as "good", with average marks of approximately 3,3.

CONCLUSIONS

In summing up, we showed that the timing of yeast collection from a fermenting tank has a significant influence on the final quality of the beer. Depending on the time chosen for separating the yeast from the beer, the characteristics of the gathered yeast thickness, the content of the individual volatile components and the sensory properties of the final product undergo changes. On the basis of the performed experiments on the industrial scale, the conclusions below were formulated:

(1) An earlier collection of the yeast directly after finishing the fermentation process leads to a maximal separation of the biomass from the beer and thus retains the flocculation properties of the yeast and improvements in its filtration properties.

(2) The beer from which the biomass of yeast was separated early was characterized by a significantly higher amount of acetaldehyde in comparison to the beers from which yeast was collected in a later period.

(3) Lower concentrations of undesirable ingredients of beer (diacetyl and 2,3-pentanedione) and

higher concentrations of ethyl acetate and isoamyl acetate were characteristics of the batches of beer in which the yeast in was left for longer periods in the fermenting tanks.

(4) The inhibition of biochemical changes directly after the fermentation process as a result of earlier collection of the yeast negatively influenced the taste and aromatic bouquet of the produced beer.

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