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The effect of yeast generations on fermentation, maturation and volatile compounds of beer

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Abstract: The aim of the study was to determine the effect of yeast generations on fermentation and maturation processes, the content of volatile compounds of beer and viability and vitality of yeast biomass on an industrial scale. The experiments with fermentation and maturation were performed in fermentation tanks. The wort was aerated with sterile air. Yeast (*S. pastorianus*) bottom fermentation was used in fermentation. For pitching four generations (passages) of yeast were used as follows: 1st, 2nd, 3rd and 4th generation. The processes of fermentation and maturation were carried out in the same technological conditions (temperature and pressure). During fermentation and maturation, the changes in the content of the extract, yeast growth and vitality and selected volatile compounds like esters, alcohols and carbonyl compounds were investigated. With the increase in the number of yeast generations, especially from the 2nd generation used in the fermentation process, the content of acetaldehyde and esters increased. Despite the slight differences between generations, the changes are statistically significant. The content of diacetyl is stable for the 1st, 2nd and 3rd generation and higher for the 4th generation. Diversified yeast generations used in the process of fermentation did not affect significantly the final quality of beer.

Keywords: wort; yeast generation; fermentation; volatile compounds; beer

After fermentation, yeast biomass is cropped from green beer and stored until it is required for use in subsequent fermentations in a process known as serial repitching. During serial repitching, the yeast cell is transferred through several physiological states: from active growth and division during propagation and the early stages of fermentation to stationary phases during fermentation, culminating in a prolonged stationary phase during storage in a fermenter cone (Jenkins et al. 2003).

After brewery fermentation, yeast is harvested from the fermentation vessel and should quickly be re-inoculated into a fresh wort batch. In some breweries, a bottom brewing yeast culture is used 2–3 times, while in others, 7–9 fermentations of wort at similar original gravity are used. Lager yeast can even be reused up to 20 times (Powell et al. 2003; Kordialik-Bogacka & Diowksz 2013).

During serial repitching, the physiology of yeast may deteriorate, and microbial contamination can occur. The physiological state of yeast prior to pitching determines the consistency of fermentation and product quality (Axcell & O'Connor-Cox 1996).

In breweries, yeast is reused in consecutive fermentations ("serial repitching"). In the high cell density (HCD) fermentation, increased amounts of propagated yeast are produced, generally making the propagation process even more expensive. A higher cell concentration

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in the initial phase of fermentation results in a lower build-up of unsaturated fatty acids per cell, which causes a decrease in the yeast net growth (Verbelen et al. 2009).

In addition to pitching rate, the number of serial croppings, storage and repitching cycles (corresponding to the so-called "generation number") is also important; generation number can influence flocculation. In addition, prolonged cultivation may also lead to genotypic variability, causing changes in the geneticall-ly determined flocculation profile of a particular strain (Jin & Speers 2000; Verstrepen et al. 2003; Lodolo et al. 2008; Vidgren & Londesborough 2011).

The goal of the presented experiments was to determine the effect of yeast generation on the processes of fermentation and maturation, as well as the concentrations of the volatile compounds of beer, and the viability and vitality of yeast biomass on an industrial scale.

By comparing results obtained under laboratory conditions by other researchers with data gathered from an industrial scale plant, this paper offers a novel look at the topic under study.

MATERIAL AND METHODS

Experimental design. This study investigates the parallel process of beer production in four different cylindroconical tanks (CCT), sampled during 18 days of the production cycle. Each cylindroconical tank was filled with three brews (each batch taking 4.5 h). Total filling time for four fermenters was 18 h. High gravity worts (15.5 °P) were prepared from the same batch of malt under identical conditions. Pilsner type malt from two malt houses was used in the experiments. Infusion mashing took place between 60 and 76 °C. Afterwards, the mash was transferred to a lauter tun and boiled in a wort kettle for 60 min (Kucharczyk &Tuszyński 2018).

Sample collection started after filling the CCT and continued during the next days at the same time every day. The CCT had a total capacity of 3 850 hL with 20% headspace.

Saccharomyces pastorianus yeasts were used for fermentation. The main fermentation lasted for the first five days of the process. Yeasts for experiments were cropped from CCT during the 5th day of maturation (at a temperature of 14°C). The warm maturation lasted for 5 days at a temperature of 14 °C. Thematuration process is divided into two phases: warm maturation and lagering. After cropping the yeast was stored in YST (yeast storage tank) for maximally 4 days at a temperature of 1.3–1.8°C under overpressure 0.05 bar. After cropping the beer was cooled down to –0.7 °C (to the phase of lagering).

For pitching four generations (passages) of yeast (1st, 2nd, 3rd and 4th) were used. The yeast was pitched (the same dose for four CCT; 7 × 106 cells mL^{-1}) into the first brews, using a high-precision fully automated ABER system for the rate control. The wort was aerated by compressed, sterile air during transfer to each CCT, with the identical intensity of 10 mg O_2 L^{-1} wort. The processes of fermentation and maturation were carried out in the same technological conditions.

Analytical procedures. Measurements included the number of yeast cells in the fermenting wort and beer and the percentage of dead cells, measured using a NucleoCounter cell analyser (Chemometec, Lillerod, Denmark).

Gravity was measured using a wort and beer analyser (Beer Analyser DMA 4500+; Anton Paar, Graz, Austria) at 20 °C. The Tabarié formula was the basis for an Alcolyzer beer system.

Qualitative and quantitative analysis of volatile compounds (the identification was done on the basis of retention time) was performed using a GC 8000 gas chromatograph (Fisons Instruments, Ipswich, UK) fitted with a flame ionisation detector GC-FID for detection of acetaldehyde, ethyl acetate, sum of higher alcohols and detector GC-ECD for detection of diacetyl and 2,3-pentanedione. The column temperature was kept at 45 °C for 10 min, increased to 120 °C at 5 °C min^{-1} and then held at that temperature for 8 min, and eventually lowered to 45 °C at 15 °C min^{-1}. The temperature of the injection zone was fixed at 140 °C. The carrier gas was helium at a pressure of 65 kPa, with a flow of 4–6 mL min^{-1}. Injection of samples (0.75 mL) was performed with an HS-800 autosampler. The sample annealing temperature was 40 °C for 40 min. The temperature of the autosampler syringe was 60 °C. Concentrations were calculated using a quantitative computer program based on the calculated peak area. The sample of beer, with a volume of 2.5 mL, was placed in a vial and conditioned at 40 °C for 40 min to equilibrate the liquid and gas phase (headspace method).

The DB-WAX capillary column (60 m long, 0.53 mm internal diameter and 1 μm thick) packed with polar polyethylene glycol was used for separation. A 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, 95% dimethylpolysiloxane) was used for the determination of diacetyl and 2,3-pentanedione. 2,3-hexanedione was used as an internal standard.

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The chromatograph was calibrated once a month. Before and after each series of measurements, a comparative analysis was carried out with a beer sample used as a control (reference) batch.

Determination of specific growth rate and the period of yeast generation. According to Müller et al. (2005):

Specific growth rate of yeast (h^{-1}):

$$\mu = 2.3 \log (m_t\, m_o^{-1})\, t^{-1} \quad (1)$$

where: m_t – received amount of yeast (million cells mL^{-1}); m_o – amount of yeast added to fermentation (million cells mL^{-1}); t – time of the process (h).

Period of yeast generation (h):

$$g = t \log 2 / (\log m_t - \log m_o) \quad (2)$$

where: m_t – received amount of yeast (million cells mL^{-1}); m_o – amount of yeast added to fermentation (million cells mL^{-1}); t – time of the process (h).

Sensory analysis. A comparison test was used for sensory evaluation of bottled beer, when the test sample was compared with the reference beer profile. The beer was tested in 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 (trained nine panellists of the brewery, generally technologists and lab technicians) 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) (Kucharczyk &Tuszyński 2018).

Statistical analysis. The results presented in this work are the means of four independent experiments with the bars representing the standard deviation (SD). The data were analysed by one-way analysis of variance (ANOVA) to test the significance of different yeast generations in relation to concentrations of volatile compounds in beer and other parameters. Significant differences between the means were verified by Duncan's test ($P < 0.05$) with the use of Statistica 10 software (StatSoft Polska, Kraków, Poland).

RESULTS AND DISCUSSION

Fermentation and maturation process. Changes in the apparent extract during wort fermentation with the use of biomass from the 1st, 2nd, 3rd and 4th generations are diverse (Figure 1). In the case of yeast from the 1st and 4th generation, the average daily extract decrease was 1.9 °P – calculated during the first five days of main fermentation. The biomass of the 2nd generation caused the acceleration of the daily extract consumption by 0.2 °P per day. In turn, the 3rd yeast generation was characterised by the slower rate of fermentation (1.8 °P per day). A decrease in the apparent extract to the value 3.3 °P means the end of the fermentation process, and simultaneously the beginning of the maturation process. Due to faster fermentation for the 2nd yeast generation the maturation process started sooner by about half a day.

Previously published research from laboratory-scale experiments showed that the kinetics of fermentation depended on the multiplicity of the yeast use (Jenkins et al. 2003; Powell et al. 2003; Stingheriu 2005; Sigler et al. 2009). In the experiments performed by Sigler et al. (2009), the generation of yeast caused significantly faster (by up to 30%) fermentation than the subsequent generations. In another study (Verbelen et al. 2009) the fermentation lasted for 190, 170 and 140 h for the 1st, 2nd and 3rd generation, respectively, while subsequent generations did not cause any further acceleration. Research by Kordialik-Bogacka and Diowksz (2013), however, did not show any significant changes in fermentation rate, even with 98 subsequent generations. Similar conclusion can be drawn from data published by Powell and Diacetis (2007). In yet another experiment, Powell et al. (2003) examined the impact of the yeast population age on the rate of fermentation. Virgin cells, in relation to older populations of yeast (heterogeneous and non-virgin cells), fermented wort carbohydrates more slowly.

Characteristics of yeast viability changes. Changes in the quantity of yeast cells during beer fermentation and maturation in relation to the yeast generation used are presented in Figure 2. The number of yeast cells was

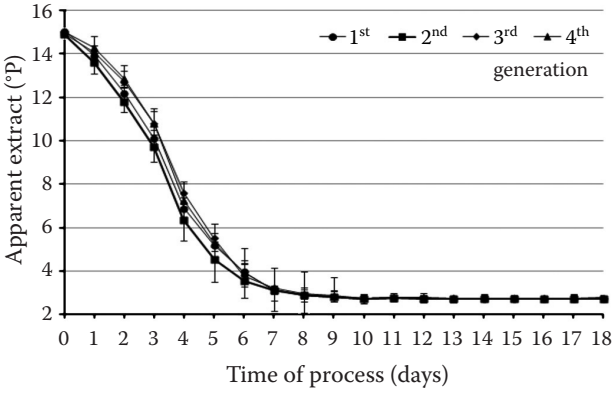

Figure 1. A decrease in apparent extract at four different yeast generations used in the fermentation process ($n = 3$)
Values are means ± standard deviation (SD)

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the highest for the 4th generation of yeast. In this case the number of cells grew fivefold (up to 37 × 10^6 of cells in 1 mL) up to the fifth day of fermentation whereas in worts fermented by yeast from the 1st, 2nd and 3rd generation, the maximum concentration of yeast cells amounted 32–35 million units in 1 mL.

The characteristics of changes in the number of yeast cells are correlated with the rate of fermentation for individual generations of yeast. The 2nd yeast generation (which influenced the faster fermentation process) was the fastest to start the flocculation process. In turn, the 1st generation yeast began to sediment at the latest – this may be caused by a lower fermentation rate. This is also influenced by the size of the yeast cells; the lower yeast generations are characterised by the smaller cell size and thus their slower process of flocculation.

The tendency of changes in the number of yeast cells around the 10th day may be caused by the fact that the tank fermenter circulation pump through the beer stream raised upwards from the cone can influence the suction of yeast into the sampling line.

Tests with the 4th generation of yeast were characterised by a higher specific rate of growth (μ) and a significantly shorter doubling time of biomass production period (Td) (Table 1). The highest generations of yeast (4th generation) used in the experiment had an impact on faster multiplication of the next new yeast generation cells by about 1–3 hours.

The impact of the yeast generation on the budding of cells and biomass increases was studied by Sigler et al. (2009), Verbelen et al. (2009) and Miller et al. (2012). Verbelen et al. (2009) showed that the highest growth of biomass (approximately 27 × 10^6 cells in 1 mL) occurred in the 1st yeast generation and then gradually decreased with subsequent uses.

Table 1. The effect of four different yeast generations used in the fermentation process on the specific rate of yeast growth (μ) and the doubling time of biomass (Td)

Generations (passages) of yeast (*n*)	μ ($1\ h^{-1}$)	Td (h)
1st	0.0159 [a]	43.56 [a]
2nd	0.0163 [a]	42.55 [a]
3rd	0.0165 [a]	42.07 [a]
4th	0.0170 [b]	40.68 [b]

Values are means of three replications; *within the same column the values bearing different superscript letters are significantly different at α = 0.05

The quantitative changes in dead yeast cells in fermenting wort and in maturating beer in relation to the generation number of yeasts are presented in Figure 3. Generally, in yeast pitching, the number of dead cells was low, ranging from 0.3% to 2.0%, depending on the multiplicity of their use. After the first day of fermentation, an increase in the dead cell count was observed, ranging well over 2% for yeast from the 1st generation, possibly due to environmental stress. At the completion of fermentation (the sixth–seventh day), the number of dead cells was within the range of 2 to 4%. At the maturation stage, the viability of yeast cells decreased and ranged from 94 to 96%. The yeast from the 1st and 2nd generation was characterised by a higher number of dead cells (8–10%). In turn, the 3rd and 4th generations were characterised by the lower content of dead cells (maximum 4%). This may indicate that the yeast of the older generation better adapts to the fer-

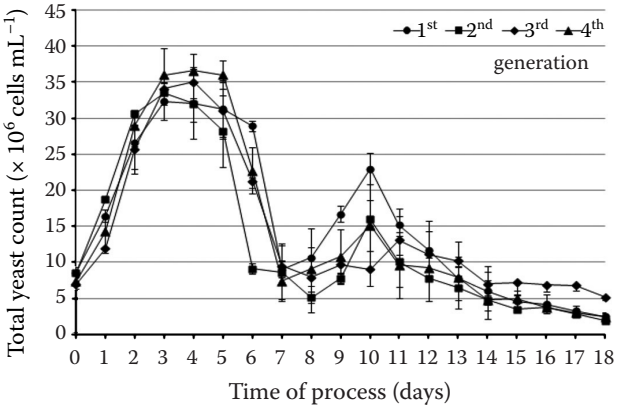

Figure 2. The effect of four different yeast generations used in the fermentation process on yeast growth (*n* = 3)
Values are means ± SD

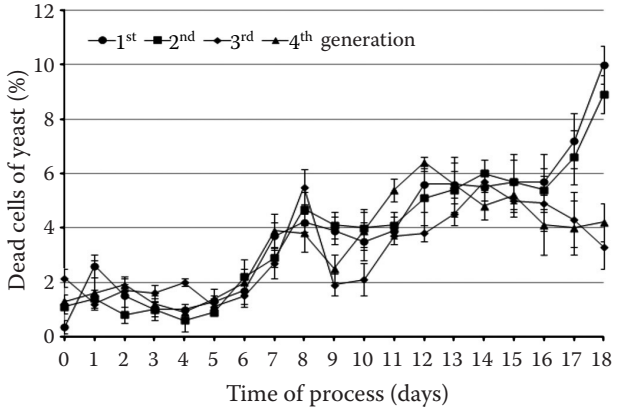

Figure 3. The effect of four different yeast generations used in the fermentation process on yeast viability (*n* = 3)
Values are means ± SD

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Table 2. The impact of four different yeast generations used for fermentation on the final concentrations of volatile compounds in beer

Flavour compounds (mg L^{-1})	Generation (passage) of yeast[y]				Significance
	1	2	3	4	
Esters					
Ethyl acetate	18.93^{ab}	18.05^{a}	18.1^{a}	19.28^{b}	0.0004
Isoamyl acetate	1.60^{b}	1.53^{a}	1.64^{b}	1.71^{c}	0.0003
Total	20.58^{c}	18.58^{a}	19.74^{b}	20.99^{d}	0.0020
Higher alcohols					
Methanol	2.02^{a}	2.22^{a}	2.95^{c}	2.55^{b}	0.0030
n-Propanol	10.68^{b}	10.65^{b}	10.25^{a}	11.12^{c}	0.0043
Isobutanol	14.78^{c}	14.45^{b}	14.10^{a}	14.58^{bc}	0.0021
Amyl alcohols	75.88^{c}	74.98^{b}	73.75^{a}	76.45^{c}	0.0018
Total	103.36^{b}	103.30^{b}	101.05^{a}	104.70^{c}	0.0110
Carbonyl compounds					
Acetaldehyde	6.08^{ab}	5.88^{a}	6.05^{ab}	6.55^{b}	0.0029
Diacetyl	0.016^{a}	0.015^{a}	0.014^{a}	0.021^{b}	0.0001
2,3-pentadione	0.017^{b}	0.016^{a}	0.016^{a}	0.016^{a}	0.0332
Total	6.12^{ab}	5.91^{a}	6.08^{ab}	6.59^{b}	0.0460
Main total	130.35^{b}	128.07^{a}	126.87^{a}	132.61^{c}	0.0410

[y]according to Duncan's test the means within columns followed by the same letter are not significantly different

mentation environment. In the present research, after fermentation, the yeast collected from each batch met the criterion for re-using, i.e. less than 5% of dead cells. In an earlier laboratory-scale study, Kordialik-Bogacka and Diowksz (2013) showed that both the multiplicity of the yeast usage and the successive accumulation of trehalose increased yeast viability. A similar tendency of change in the viability of yeast after successive uses was presented in a study by Stewart (2015). In yet another studies Jenkins et al. (2003) and Verbelen et al. (2009) indicated different tendencies, particularly in relation to the first four generations of yeast.

Beer volatile compounds. The effect of the yeast generation on the content of volatile compounds in final beer was demonstrated in our experiments (Table 2). Research in this area showed an important relationship between subsequent generations of yeast and the chemical composition of beer (Verbelen et al. 2009). The contents of acetaldehyde in beer fermented with the four generations of yeast are presented Figure 4. Our experiments performed in an industrial plant showed that the yeast of the first batch produced 30, 15 and 5% more acetaldehyde than those from the fourth, second and third fermentation cycles, respectively. At the end of fermentation, the contents of acetaldehyde in beers from all the experiments were comparable, amounting approximately to 10 mg L^{-1}. After collecting the yeast slurry, the concentration of acetaldehyde in beer was found to be 7 mg L^{-1}. During beer maturation, the content of acetaldehyde gradually decreased to the level of 5.88 to 6.55 mg in 1 L of beer in all four examined yeast generations. Our experiments clearly indicate that wort fermentation with the yeast of the1st, 2nd and 3rd and 4th generation had a significant influence on acetaldehyde content.

Based on the course of changes in the acetaldehyde content, it can be stated that with a higher fermentation rate, the resulting concentration value of this compound is lower, and vice versa. This observation is important to design beers with a shorter technologi-

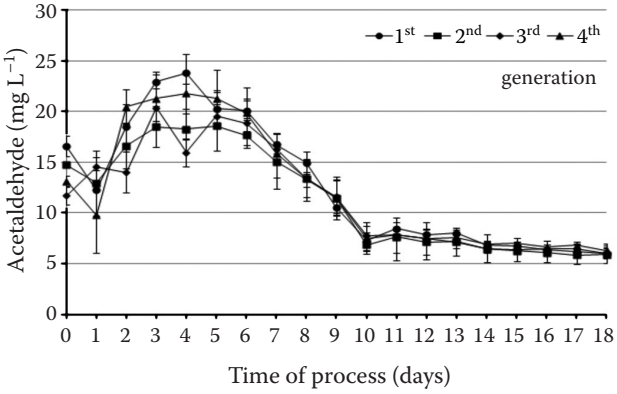

Figure 4. Formation of acetaldehyde content depending on the four different yeast generations used in the fermentation process (n = 3)

Values are means ± SD

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cal process to get the right content of acetaldehyde in a short period. The same applies to other beer volatile compounds, especially vicinal diketones.

Verbelen et al. (2009) described similar characteristic changes in the concentration of acetaldehyde in beer with subsequent generations of yeast. The authors observed an almost 50% increase in acetaldehyde content using the 4th generation of yeast as compared to the 1st generation. In contrast, Sigler et al. (2009) proved a different tendency of acetaldehyde biosynthesis with a slight concentration decrease (approximately 10%) between the 2nd and the 3rd yeast generation. These discrepancies might have resulted from different initial wort concentrations (12 and 15 °P), different yeast strains, or different degree of aeration employed in these studies.

The average content of diacetyl in the wort attenuated with the 4th generation of yeast was approximately 30% higher than with yeast from the 1st, 2nd and 3rd generations. Verbelen et al. (2009) indicated a doubled diacetyl concentration in beer fermented with reused yeast which was cropped after the first fermentation process. In the present study the doses of yeast pitching were almost threefold smaller than those applied by Verbelen et al. (2009). Small inoculum doses caused faster growth of new cells, which in turn led to faster "rejuvenation" of the population and was characterised by a higher activity of enzymes and an accelerated pace of biochemical changes as reported by Axcell and O'Connor (1996). In yet another work Sigler et al. (2009) did not observe any significant changes in the synthesis of vicinal diketones that could be attributed to the yeast generation number but rather a stable concentration of diacetyl at around 85 μg L^{-1} when the 2nd and the 3rd yeast generations fermented 12 °P worts.

Among esters, the amounts of ethyl acetate and isoamyl acetate were analysed. The results of our study demonstrated that the increase in the generation number from the 1st to 2nd generations resulted in a decrease in ethyl acetate and isoamyl acetate concentrations. The yeast from the 4th generation, however, produced an approximately 7% increase in the concentrations of ethyl acetate and isoamyl acetate. The ester content profiles in relation to the yeast generation number were presented in research by Kordialik-Bogacka and Diowksz (2013), Sigler et al. (2009) and Verbelen et al. (2009).

Research conducted by Kordialik-Bogacka and Diowksz (2013) showed that during repitching, the yeast strain was the most important factor that influenced the content of esters. One of the investigated strains produced constant amounts of ethyl acetate and isoamyl acetate. Verbelen et al. (2009), on the other hand, reported that the ester concentration decreased to the 4th yeast generation, and then it increased.

Sensory evaluation. The experiments showed that the application of different batches of yeast (from the 1st to the 4th generation) in beer fermentation did not have a noticeable impact on the sensory quality of the final product. The research showed that the content of the main flavour components that always greatly influence the taste quality of beer was synthesised at a suitably steady low level and therefore all the beers studied were assessed as "good" on the scale ranging from 3.2 to 3.3 points. Further research is needed for a reference sensory assessment to determine the chemical composition of beer, especially volatile compounds as a function of the applied yeast generations, the strain of yeast and the technological conditions of beer fermentation and maturation.

CONCLUSIONS

The selection of an appropriate strain of yeast for beer production is a basic condition for replicating the taste of the final product. The yeast studied here showed stable genetic and physicochemical features, allowing the prolonged use of biomass in subsequent fermentations.

The maximum number of applied generations of yeast is determined by each brewery and primarily depends on the applied strains of yeast, brewery equipment, and applied technology, type of beer, and microbiology, as well as beer storage cellar conditions in the fermenting plant. On the basis of the research performed under industrial conditions, we can state that the use of biomass for four cycles has no negative influence on the physicochemical composition and sensory features of beer. Furthermore, the yeast from the 4th generation displayed a few technological advantages over the biomass from earlier generations.

Literature reports and practical experience indicate that the prolonged use of yeast (more than four times) is very economically beneficial. Higher generations no longer show increased biomass growth and therefore beer losses are smaller.

The novelty of the article is that these experiments were conducted in industrial conditions and the obtained results may be a material for comparison with similar trials on a laboratory and other industrial scale.

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REFERENCES

Axcell B., O'Connor-Cox E. (1996): The concept of yeast vitality – an alternative approach. In: Proceedings 24th Convention of the Institute of Brewing, Asia Pacific Section. Institute of Brewing, Singapore, Indonesia: 64–71.

Jenkins C., Kennedy A., Hudgson J., Thurston P., Smart K. (2003): Impact of serial repitching on lager brewing yeast quality. Journal of the American Society of Brewing Chemists, 61: 1–9.

Jin Y.L., Speers R.A. (2000): Effect of environmental conditions on the flocculation of *Saccharomyces cerevisiae*. Journal of the American Society of Brewing Chemists, 58: 108–116.

Kordialik-Bogacka E., Diowksz A. (2013): Physiological state of reused brewing yeast. Czech Journal of Food Sciences, 31: 264–269.

Kucharczyk K., Tuszyński T. (2018): The effect of temperature on fermentation and beer volatiles at industrial scale. Journal of the Institute of Brewing, 124: 230–235.

Lodolo E., Kocks J., Axcell B., Brooks M. (2008): The yeast *Saccharomyces cerevisiae* the main character in beer brewing. FEMS Yeast Research, 8: 1018–1036.

Miller K., Box W., Boulton Ch., Smart K. (2012): Cell cycle synchrony of propagated and recycled lager yeast and its impact on lag phase in fermenter. Journal of the American Society of Brewing Chemists, 70: 1–9.

Müller A.G., Manger HJ., Lietz P. (2005): The yeast in the brewery. Management pure yeast cultures propagation. VLB Berlin, Germany: 135–137. (In German)

Powell Ch., Diacetis A. (2007): Long term serial repitching and the genetic and phenotypic stability of brewer's yeast. Journal of the Institute of Brewing, 113: 67–74.

Powell Ch., Quain D., Smart K. (2003): The impact of brewing yeast cell age on fermentation performance, attenuation and flocculation. FEMS Yeast Research, 3: 149–157.

Sigler K., Matoulkova D., Dienstbier M., Gabriel P. (2009): Net effect of wort osmotic pressure on fermentation course, yeast vitality, beer flavor, and haze. Applied Microbiology and Biotechnology, 82: 1027–1035.

Stewart G. (2015): Seduced by yeast. Journal of the American Society of Brewing Chemists, 73: 1–21.

Stingheriu R. (2005): Preservation method of yeast and performance in beer fermentation. Journal of Agroalimentary Processes and Technologies, 2: 337–344.

Verbelen P., Tinne E., Dekoninck E., Van Mulders S., Delvaux F., Delvaux F. (2009): Stability of high cell density brewery fermentations during serial repitching. Biotechnology Letters, 31: 1729–1737.

Verstrepen K., Derdelinckx G., Verachtert H., Delvaux F. (2003): Yeast flocculation: what brewers should know. Applied Microbiology and Biotechnology, 61: 197–205.

Vidgren V., Londesborough J. (2011): 125th anniversary review: Yeast flocculation and sedimentation in brewing. Journal of the Institute of Brewing, 117: 475–487.

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