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THE INFLUENCE OF FERMENTATION TEMPERATURE ON THE GROWTH OF YEAST BIOMASS IN BEER PRODUCED ON AN INDUSTRIAL SCALE®

Wpływ temperatury fermentacji na przyrost biomasy drożdży w piwie produkowanym w technologii wielkozbiornikowej®

The article presents the results of the influence of different fermentation temperature on biomass growth in beer produced on an industrial scale. Worts were aerated with sterile air in an amount of 10 mg per dm³. Yeast pitching rate were the same for all processes tested – 7 mln cells per cm³ wort.

The examined parameter was a variable fermentation temperature: 8.5; 10 and 11.5°C. Other parameters of the beer fermentation and maturation process in tank fermenters were carried out under the same technological conditions.

Studies have shown that a varied fermentation temperature has a significant impact on the growth of yeast biomass in the fermentation process. As the fermentation temperature increased, the amount of biomass multiplied increased. The greater number of new yeast cells contributes to greater beer loss during the fermentation of the wort.

Key words: wort, tankfermentor, fermentation temperature, yeast biomass, extract losses.

Celem artykułu jest przedstawienie wyników badań dotyczących wpływu temperatury fermentacji na szybkość procesu fermentacji oraz zaniki piwa wytwarzanego w technologii wielkozbiornikowej. Doświadczenia wykonano w warunkach przemysłowych – fermentacja i dojrzewanie w tankofermentorach o pojemności 3800 hl. Do brzeczki dodawano drożdże zebrane po drugiej fermentacji (trzeci pasaż) w tej samej ilości do każdego tankofermentora. Brzeczkę napowietrzano sterylnym powietrzem w ilości 10 mg na dm³. Procesy fermentacji głównej przebiegały w trzech badanych temperaturach: 8,5; 10 i 11,5°C. Proces dojrzewania piwa w wymienionych tankofermentorach prowadzono w tych samych warunkach technologicznych. Doświadczenia wykazały, że zróżnicowana temperatura fermentacji ma istotny wpływ na przyrost biomasy drożdży w piwie. Wraz ze wzrostem temperatury fermentacji zwiększała się ilość namnożonej biomasy. Większa ilość nowych komórek drożdży przyczynia się do większej straty piwa na etapie fermentacji brzeczki.

Słowa kluczowe: brzeczka piwna, tankofermentor, temperatura fermentacji, biomasa drożdży, zanik piwa.

WPROWADZENIE

Beer is the world's most widely consumed and probably the oldest alcoholic beverage, it is the third most popular drink overall, after water and tea. The production of beer is called brewing. Each ingredient has its own function. Barley provides the starch which is converted to maltose and other sugars, and finally to alcohol and carbon dioxide [5].

The principal raw materials used to brew beer are water, malted barley, hops, and yeast.The brewing process involves extracting and breaking down the carbohydrate from themalted barley to make a sugar solution (called "wort"), which also contains essential nutri-ents for yeast growth, and using this as a source of nutrients for "anaerobic" yeast growth. During yeast fermentation, simple sugars are consumed, releasing energy and producing ethanol and other flavoring metabolic by-products.

The major biological changes that occur in the brewing process are catalyzed by naturally produced enzymes from barley (during malting) and yeast [4].

Yeast has the ability to adjust its metabolism to aerobic as well as to anaerobic conditions. The yeast doubles or triples its mass during fermentation. For the build-up of cell substance (proteins and enzymes) the yeast needs mostly amino acids, which are taken either from the fermentation substrate or synthesized by itself. Besides proteins, lipids are also synthesized for yeast propagation because they are important components of the cell wall, and are needed for the uptake of nutrients. For the synthesis of these lipids from acetyl coenzyme A, molecular oxygen is needed; after lautering, wort

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itself contains only few lipids. Finally, the yeast also requires minerals for the stabilization of its enzyme systems [3].

The wort transforms into beer during alcoholic fermentation and maturation, which are the longest processes in brewing. The primary fermentation lasts between 3–6 days and the maturation – up to 2 weeks depending on the fermentation type and the used equipment. The ethanol fermentation occurs as a result of enzymatic activity of the yeast at Embden-MeyerhofParnas pathway, which leads to glucose conversion to pyruvate. Under anaerobic conditions the yeasts convert pyruvate to ethanol and CO_2. In aerobic conditions, yeasts consume sugars, mainly for biomass accumulation and CO_2 production (Boulton and Quain, 2001) [7]. The Balling's equation suggests, that from 2.0665 g wort extract, we received 0.11 g yeasts biomass and all sugars in the wort are fermentable monosaccharaides [7].

The aim of the article is to present the results of the impact of different wort fermentation temperature carried out in industrial conditions on the growth of yeast biomass and losses of beer.

MATERIALS AND METHODS

Experimental design

This study investigates the parallel process of beer production in three different cylindro-conical tanks (CCT), sampled during 18 days of the production cycle. Each cylindro-conical tank was filled with three brews (each batch taking 4.5 h) Total filling time for three fermenters was 13.5 h. High Gravity worts (15.5°P) were prepared from the same batch of malt under identical conditions.

Sample collection started after filling the CCT and continued during the following days at the same time every day. Sampling used a device equipped with an installed small pump working in a closed loop, enabling to be taken at vessel. The sampling point was located above the cone, 5 m from the bottom of the tank The CCT had a total capacity of 3850 hl with a 20% headspace. In order to obtain representative samples, the circulation pump was kept running during the process, but was switched off (approximately 24 hours) before yeast cropping.

In this work, a third generation bottom fermenting yeast was used and stored in the same yeast storage tank (YST). Yeast was pitched using ABER system for rate control. The wort was aerated with sterile air. The fermentation was performed at (Fig. 1): 8.5; 10 and 11.5°C. The beer maturation was carried out in the same technological conditions.

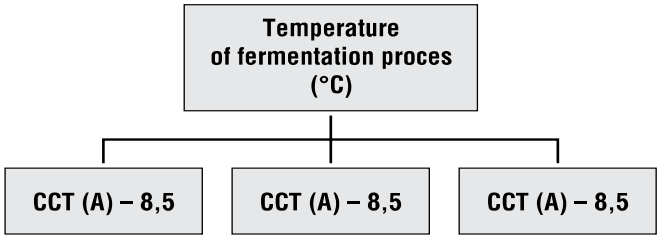

Fig. 1. Temperature of the wort fermentation.
Rys. 1. Temperatura fermentacji brzeczki.
Source: The own study
Źródło: Badania własne

Fermentation analysis

The measurement of the volume of collected biomass was obtained from the reading of flow meters placed in the yeast collection line from individual tank fermenters. Volume registration was carried out automatically using an automatic production program.

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

Measurement of biomass and yeast vitality

The total yeast concentration and the content of dead cells during fermentation and maturation of beer and in yeast slurry was determined using the NucleoCounter YC-100. This system identifies and counts single cells with stained DNA. A fluorescent microscope built into the device consists of light-emitting diodes, very high emission filters, optics, and a CCD camera. Propidium iodide combined with coloured DNA begins to emit red fluorescent light. The NucleoCounter is equipped with advanced software for final image analysis.

Measurement of extract losses

The disappearance of beer, also known as the loss of extract expressed as a percentage, was calculated on the basis of the difference in the amount of fermented wort and the obtaining beer to the initial amount of wort.

Statistical analysis

The results presented in this work were the average of three independent experiments with the bars representing the standard deviation. The data was analysed by one-way analysis of variance (ANOVA) to test the significance of the different fermentation temperatures on the fast of fermentation and beer losses produced on industrial scale. Significant differences between the means were verified by Duncan test ($P < 0.05$). Analyses of variance ANOVA were made with the use of Statistica v.10 (StatSoft Polska, Kraków, Poland).

RESULTS AND DISCUSSION

Fermentation temperature is one of the most important process parameters of beer production. Higher fermentation and maturation temperature of processes are used in industrial production using tank fermentors – differently to classical technology.

The wort fermentation and beer maturation temperatures in the tested fermenters are presented in Fig. 2. The wort temperature of pitching the yeast in each case was 8.5°C. Then, depending on the preset fermentation temperature (8.5°C, 10.0°C, 11.5°C), the special Fermos program controlled the conditions in each tank fermenter through a remote-controlled refrigeration system. To ensure a predetermined temperature (set point) for each process (experiment), during the first days of fermentation (from the 4th to the 6th day), after droping the apparent extract to 7.8°Blg (set point in the Fermos program), fermenter cooling was turned off to raise the temperature to 15°C - the first stage of maturation.

The slow increase in temperature (an increase of 1°C corresponded to a reduction of the extract by 1°Blg) lasted

from 2 to 5 days, depending on the intensity of the main fermentation.

Diacetyl content (< 35 µg per dm^{-3}) was assumed to be the indicator of the end of the first stage of maturation at 15°C. Then the contents of the tankfermentor were cooled in about 4 days to a temperature of about -0.7°C and the lagering process was carried out (further maturing), which lasted a minimum of 3 days. Based on the course of the graph line (Fig. 2), it can be concluded that the increase in fermentation temperature from 10 to 11.5°C caused acceleration of fermentation, and thus the reduction of the required process time by 24 hours. In turn, the decrease in temperature from 10 to 8.5°C slowed down the process by one day. It should be noted that the speed of the process is mainly the result of an intense increase in yeast biomass due to the higher fermentation temperature.

Figure 3 shows the formation of yeast cell content during the fermentation process of wort, depending on the fermentation temperature (8.5; 10 and 11.5°C).

The obtained results indicate that the cell number reached the highest values at 11.5°C. By day 5 of the process, the number of cells had increased more than 6-fold (to 42 million CFU in cm^3). Increasing the temperature by 0.5°C caused a multiplication of the amount of yeast density collected from the fermentation tank. Figures 4 and 5 show the appropriately pumped quantity of yeast slurry and the percentage increase in biomass, depending on the fermentation temperature.

After the set yeast collection time (100 ± 4 hours from the beginning of maturation), biomass was discharged (pumped) from tank fermenters to yeast tanks.

During the fermentation process, yeast uses the extract available in the wort to produce ethyl alkohol, carbon dioxide, and fermentation by-products. From 100 g of fermenting sugars, it has been proven that about 6-7 g is used for the growth of yeast biomass [1].

The conducted research measured the increase in yeast biomass after each fermentation. Significant differences were found in the biomass growth, depending on the change in the parameters studied.

The effect of temperature on the increase in yeast propagation is comparable to the temperature coefficient of transformation (Q10) determined by Arrhenius. The yeast biomass doubles when the fermentation temperature is increased by 10°C until the maximum and optimal temperature is reached 25°C for Saccharomyces carlsbergensis [6]. The assessment of the effect of process temperature on cell budding and biomass growth was the goal of research, among others Claro et al. [2] and Saerensa et al. [8]. The authors showed a close relationship between temperature and viability as well as the rate of yeast propagation.

Our experiments showed that at 11.5°C, there was an over 6-fold increase in cell number. The volume

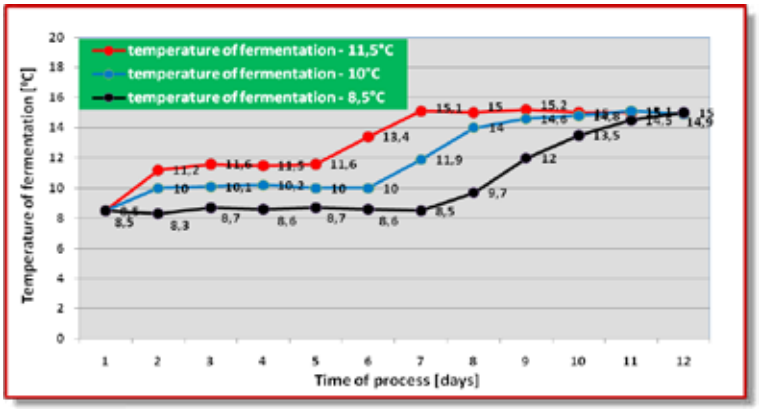

Fig. 2. Course of temperature in the process of wort fermentation.
Rys. 2. Kształtowanie się temperatury w procesie fermentacji brzeczki.
Source: The own study
Źródło: Badania własne

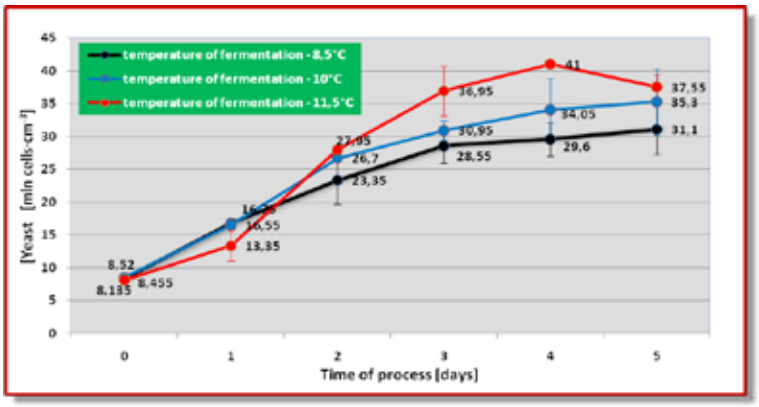

Fig. 3. The number of yeast cells in the fermenting wort, depending on the fermentation temperature.
Rys. 3. Liczba komórek drożdży w fermentującej brzeczce w zależności od temperatury procesu.
Source: The own study
Źródło: Badania własne

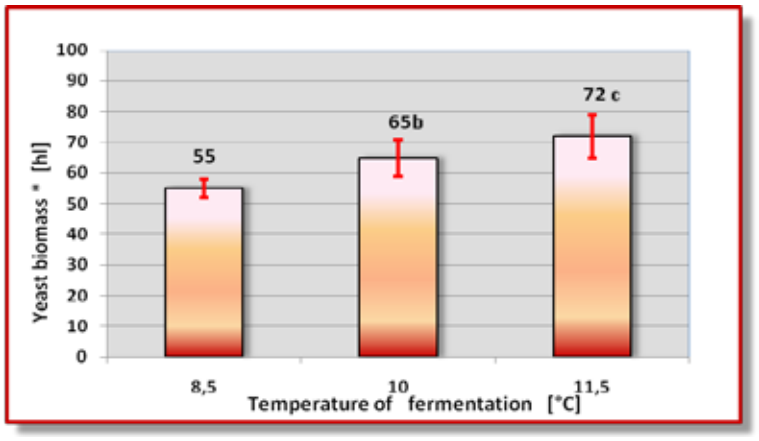

Fig. 4. Volume of yeast slurry collected depending on fermentation temperature. (*slurry with biomass concentration – 109 mln cells·cm-3)
Rys. 4. Objętość gęstwy drożdżowej w zależności od temperatury fermentacji. (*gęstwa drożdżowa o koncentracji - 10^9 mln komorek·cm^{-3})
Source: The own study
Źródło: Badania własne

increase in biomass at 8.5, 10 and 11.5°C was 245, 290 and 355%, respectively. Both temperature and aeration are the main factors determining yeast growth and biomass efficiency.

Important indicators of the profitability of the brewery are loss of beer, currently converted into „extract losses", which is determined as the percentage of extract lost during the entire production process.

Extract losses are mainly associated with the disappearance of small amounts of beer in technological lines and the increase in yeast biomass, which during fermentation consumes part of the carbohydrates contained in the wort to build cell structures during reproduction.

Figure 6 illustrate the loss of extract depending on the factors examined. As the fermentation temperature increases the extract losses also increase. At the fermentation temperature of 8.5°C, its average disappearance was 1.03%, while at 11.5°C the loss of extract was already 2.5 times higher. This state of affairs is mainly due to the increased biomass of yeast.

CONCLUSION

1. A significant effect of fermentation temperature on the growth of yeast biomass during the fermentation process was found. At higher fermentation temperatures, more new yeast biomass is produced.
2. Studies have shown the start of sedimentation of yeast cells from the fifth day of the fermentation process.
3. Greater multiplication of yeast biomass at higher temperatures contributes to higher losses of fermented wort (extract losses) and simultaneously produced beer.

WNIOSKI

1. Stwierdzono istotny wpływ temperatury fermentacji na przyrost biomasy drożdży podczas procesu fermentacji. Wyższe temperatury fermentacji powodują większe namnożenie młodych komórek drożdży.
2. Badania wykazały, że w doświadczeniach prowadzonych w warunkach przemysłowych, proces flokulacji drożdży rozpoczyna się od 5 dnia procesu fermentacji.
3. Większe namnażanie biomasy drożdży w wyższej temperaturze przyczynia się do większych strat fermentowanej brzeczki (strata ekstraktu) i jednocześnie wyprodukowanego piwa.

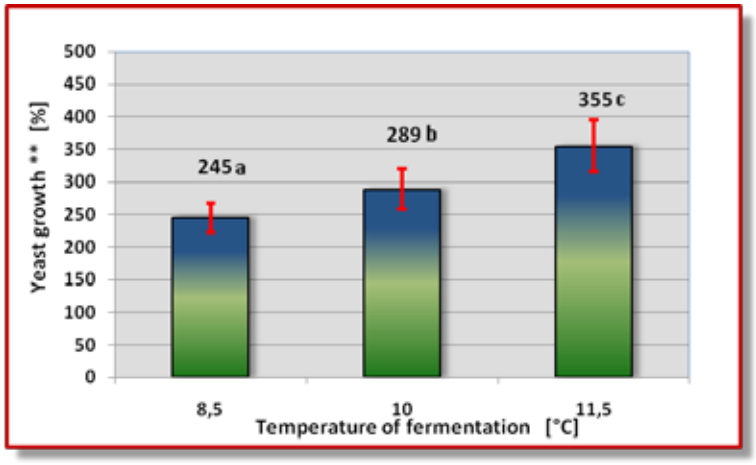

Fig. 5. Percentage increase of yeast growth depending on fermentation temperature. (ratio of biomass after fermentation to the amount of pitching yeast per biomass concentration - 10^9 mln cells·cm^{-3})**

Rys. 5. Procentowy przyrost biomasy drożdży w zależności od temperatury fermentacji. (współczynnik ilości uzyskanej biomasy drożdży po fermentacji do ilości zadanych drożdży w przeliczeniu na koncentrację biomasy - 10^9 mln komórek·cm^{-3})**

Source: The own study

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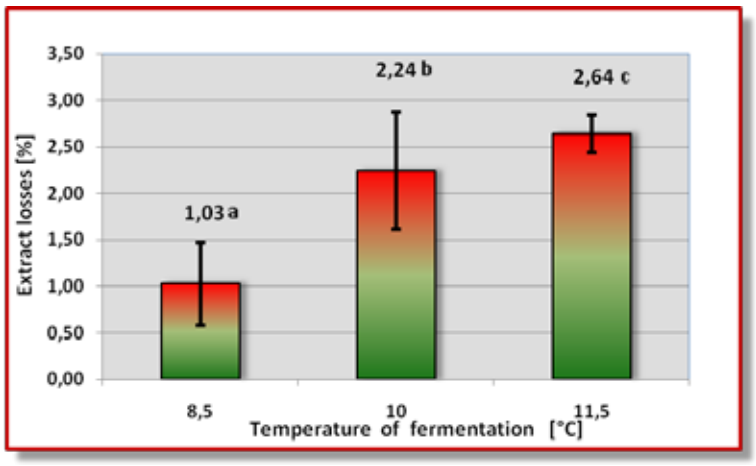

Fig. 6. Extract losses depending on temperature of fermentation.

Rys. 6. Straty ekstraktu w zależności od temperatury fermentacji.

Source: The own study

Źródło: Badania własne

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