When fermenting beer, in addition to the formation of basic, secondary, and by-products, which largely determine the taste and aroma of the beverage, yeast is accumulated. Such yeasts can be reused in subsequent serial repitching, which allows cost savings compared to yeast cultivated for each fermentation.

This paper investigates the influence of yeast generation on the progress of the wort fermentation process and the quality of beer obtained, specifically in high gravity brewing.

It was established that the increase in the number of yeast generation in the range from the first to the fourth generation causes a slight acceleration of the fermentation process of high gravity wort, while at higher values of the fermentation generation number it slows down. Yeasts secreted after six or four serial wort fermentations with a gravity of 15 °P or 18 °P, respectively, have a good physiological state and can be reused. The yeast generation number does not significantly affect ethanol content and sensory beer quality indicators. However, the use of yeast of the seventh and eighth generation causes a slight increase in the pH and color of high gravity beer, the appearance in the aroma of noticeable extraneous shades – yeast tone, tone of young beer.

As wort gravity increases, the number of yeast reuse cycles decreases, which is a consequence of the influence of various stress factors, specifically high osmotic pressure, and increased ethanol levels. Therefore, for the fermentation of wort with a gravity of 15 °P, it is recommended to use yeast up to five generations, for 18 °P wort – to four generations. To obtain beer with appropriate physicochemical parameters and high sensory evaluation in high gravity brewing technology, it is advisable to ferment wort with a gravity of 15 °P with yeast for up to five generations.

Keywords: high gravity brewing, yeast generation, fermentation dynamics, physiological state, beer quality

1. Introduction

A key criterion that plays an increasing role in the brewing market is the quality of beer. Components that determine the sensory quality of the beverage include aroma, taste, saturation, bitterness, clarity, foam, and color. However, the most important features are the taste and aroma of beer, depending on many factors, specifically the quality of malt and hop raw materials, as well as the parameters of technological processes [1]. A significant role in the formation of the sensory profile of beer belongs to yeast, which ferments wort sugars and turns them into flavoring compounds of beer.

High gravity brewing (13–18 °P) has become an attractive alternative to traditional methods as it has a wide range of advantages, from improving the cost-effectiveness of the process to reducing environmental impact [2]. However, under the conditions of high gravity brewing, yeast is affected by various stress factors, specifically high osmotic pressure, and high ethanol levels. That negatively affects its productivity and often leads to slow or incomplete fermentation. In addition, unwanted changes were detected in the taste and aromatic profile of high gravity beer [2–4].

In this regard, determining those conditions that ensure the physiological stability of yeast is a relevant task that could make it possible to approach the solution to one of the most important issues in the world brewing related to instability in the sensory characteristics of beer [5].

During the fermentation of beer, in addition to the formation of basic (alcohol and carbon dioxide), secondary, and by-products, which largely determine the taste and aroma of the beverage, yeast is accumulated in the fermentation apparatus.

In this case, its biomass increases three to four times. Such yeasts can be reused in subsequent serial repitching. That makes it possible to achieve cost savings compared to using yeast cultivated for each fermentation. After all, the cultivation of yeast is a very time-consuming process, consisting of several stages and includes costs, specifically, for the sterilization and cooling of wort.

In production, the middle layer of yeast is reused, which has high fermentation activity [6]. After fermentation, yeast biomass is separated from the young (green) beer and stored until it is needed for use in subsequent fermentations. In some breweries, yeast culture is used two or three times, in others – for seven to nine consecutive wort fermentations of the same original gravity, and even up to 20 times [7].

Specially cultivated yeasts are called yeasts of zero generation (G0), after their use for fermentation and separation upon completion of fermentation – first generation yeast (G1), after their reuse in the next fermentation cycle – yeast of the second generation (G2), etc. [8]. The number of yeast separation, storage, and reuse cycles is termed a generation number. After washing with water, yeast is a thick pasty mass, which contains...
up to 12.5 % of solids and 400 million cells per 1 cm³. Such yeast can be used immediately after washing or stored for up to two days under a layer of water at a temperature of 1–2 °C.

Adjusting the number of serial repitching of yeast is essential to ensure the required degree of fermentation and the quality of the resulting product. Consecutive fermentations of wort with a gravity of 10 °P and 15 °P at a temperature of 9 °C were investigated in [9]. According to the results of research, no changes in the ability of yeast to ferment up to eight to ten generations were detected. However, in high gravity brewing technology, the fermentation of wort with a gravity of 13–18 °P is carried out at higher temperatures in order to intensify the process. Therefore, further study is required into the effect of yeast generation on the fermentation of higher gravity wort at higher temperatures.

Similar results were reported in [10], whose authors studied a series of industrial fermentations. The number of yeast generations (up to 13) did not affect any of the process parameters. The authors concluded that the practice of disposing of yeast after 10 fermentations (and the associated costs of yeast cultivation) may be questioned and deserve further study. However, the cited work does not specify any indicators for the fermented wort. Such results can characterize the fermentation that is carried out using traditional technology.

The authors of [7] investigated consecutive fermentations of 15.5 °P wort at a temperature of 14 °C under industrial conditions, using yeast from the first to the fourth generation. It was found that the use of yeast for four cycles has no negative impact on the physicochemical composition and sensory features of beer. In addition, the yeast of the fourth generation showed several technological advantages over the biomass of previous generations. However, the cited work does not investigate the impact of a larger number of generations, which can be cost-effective.

During high gravity fermentation, yeast is exposed to several stressful conditions, such as high osmotic pressure caused by a high concentration of sugars at the beginning of fermentation, and stress caused by an increased concentration of ethanol at the end of fermentation [11]. As a result, there may be a decrease in the fermentation rate and incomplete fermentation in wort with a gravity of more than 18 °P. Such fermentations lead to high residual sugars, mainly maltotriose and maltose, a decrease in the amount of ethanol formed, and changes in the taste and aromatic profile of the beer.

The influence of higher wort gravity on the duration of fermentation at a temperature of 12 °C was investigated by the authors of [12]. Three consecutive fermentations were carried out: 12°P/12°P/12°P, 16°P/16°P/12°P, and 20°P/20°P/12°P. The results are quite different from those reported in [7, 9, 10]. Specifically, the authors of the cited work observed a decrease in the fermentation rate by 86 %, 65 %, and 39 % for second-generation yeasts in 20 °P, 16 °P, and 12 °P wort, respectively.

Similarly, a noticeable (39 %) slowdown in fermentation was detected after repitching the yeast of the third generation of all studied groups in 12 °P wort. This means that the increase in the duration of fermentation is more due to the serial use of yeast than to the gravity of the wort. However, the return of G3 yeast to the normal wort gravity of 12 °P caused a significant reduction in fermentation compared to G2 yeast, which shows that the gravity of wort still has an impact.

The authors of work [13] also report on the extended duration of fermentation with the participation of yeast of the second and third generation and explain this fact by reducing the viability of yeast.

Thus, the effect of repeated yeast seeding on their productivity, the state of yeast after fermentation, and the quality of the beer obtained is an important but insufficiently studied process.

Specifically, the review of studies in this area indicates the contradiction of the conclusions obtained by the above authors. This, among other things, is due to particular research under different conditions. In this regard, studying the effect of the reuse of yeast in high gravity brewing (at elevated values of wort gravity and process temperature) is relevant and of practical importance.

### 3. The aim and objectives of the study

The purpose of the study is to determine the effect of yeast generation on the progress of wort fermentation, specifically in high gravity brewing, and the quality of the beer received. Application for yeast fermentation of the specified number of generations would ensure stable productivity and obtain homogeneous beer with an appropriate taste and aromatic balance.

To accomplish the aim, the following tasks have been set:
- to determine the generation of yeast, the use of which, specifically high gravity wort, provides a stable speed and duration of fermentation;
- to determine those yeast generations that, according to the physiological state, meet the criteria for their further use in the next serial repitching;
- to determine those yeast generations whose participation produces beer with stable physicochemical quality indicators;
- to determine those yeast generations whose participation yields beer with stable corresponding sensory characteristics;
- to determine the recommended number of yeast generations for their effective use, specifically in high gravity brewing, to ensure stable fermentation productivity and homogeneous quality of the resulting beer.

### 4. The study materials and methods

This study used the Saflaqer W-34/70 yeast race. The yeast was cultivated in 11 °P wort at a temperature of 25 °C in three stages to receive the zero-generation yeast (G0). The G0 yeast was used to ferment wort with a gravity of 11 °P, 12 °P, 15 °P. Upon completing the process, the yeast of generation G1 was separated from young beer to use for the fermentation of wort of the same gravity in the subsequent fermentation cycle. The fermentation of 18 °P wort involved the G2 yeasts, separated after the fermentation of 15 °P wort. It was believed that this could ensure the gradual adaptation of yeast cells to the conditions of high osmotic pressure. After the completion of fermentation, the yeast of generation G3 was separated. In total, eight yeast generations were separated and studied. Fermentation and maturation processes were carried out under the same technological conditions, specifically, fermentation – in the temperature range of 12–15 °C for 4–7 days (depending on the gravity of the wort and yeast generation), post-fermentation – at a temperature of 0–2 °C for 10 days.
Wort gravity (°P) and apparent beer extract (°P) were measured by the areometric method (MEBAK. B 590.09.900. Apparent extract), ethanol content (% vol/vol) – by a pycnometer, after the distillation of beer (MEBAK. B 590.10.024. Original wort, extract and alcohol – by distillation (official method)), the pH of beer – by potentiometry (MEBAK. B 590.00.040. pH measurement), coloration was determined by the colorimetric method (MEBAK. B 400.03.115. Wort and beer color – colorimetric) and expressed in units (1 unit equals 1 cm² 0.1 n. of iodine solution per 100 cm³ of water).

To determine the viable cells, yeast preparations were painted with methylene blue (viable cells remained colorless), and, to determine cells with glycogen, with Lugol’s solution. Cells (at least 300) were calculated using the XS-5510 microscope (Optics&Electronics) and expressed as a percentage of the total population.

The sensory evaluation of beer was determined according to the results of open tasting by the method of point-score evaluation. The quality components and their descriptors are given in Table 1. The points received when evaluating each indicator were summed up. It was believed that beer of excellent quality has a score of 18–20 points, good – 14–17, satisfactory – 8–13, unsatisfactory – less than 8 points.

The study was carried out at the production facilities and a laboratory of LLC «Mykulynetskyy Brovar».

### 5. Results of studying the impact of yeast generation and wort gravity on the progress of fermentation and the quality of beer

#### 5.1. Fermentation

The use of yeast of different generations affects (for an individual generation – significantly) the fermentation rate and, as a result, determines the duration of the process (Fig. 1). Specifically, the duration of wort fermentation with a gravity of 11 °P, used in traditional brewing, is four days in the case of the use of yeast generations G0–G6. However, with the participation of the G7 and G8 yeast, the fermentation extends to five and six days, respectively. Similar results are characteristic of the fermentation of 12 °P wort.

In contrast, during the fermentation of high gravity wort with a gravity of 15 °P, a significant decrease in the fermentation rate is already characteristic of the G6 yeast generation, and, during the fermentation of 18 °P wort – already with the participation of the G5 yeasts (Fig. 2). As a result, the fermentation of high gravity wort took more time, specifically, the fermentation of 15 °P wort with the G0–G6 yeasts lasted five days and with the yeast generations G7 and G8 – six days. The fermentation of 18 °P wort lasted six days with the use of yeasts G3 and G4, and seven days with the participation of the G5–G8 yeasts.

| Quality indicator | Sensory characteristics | Points earned |
|-------------------|-------------------------|---------------|
| **Clarity**       | clear with gloss without suspended particles | 3 (excellent) |
|                   | clear without gloss, single small suspended particles | 2 (good) |
|                   | weak opalescence         | 1 (satisfactory) |
|                   | significant opalescence, turbid | 0 (unsatisfactory) |
| **Color**         | corresponds to the type of beer, is at the minimum level for this type of beer | 3 (excellent) |
|                   | corresponds to the type of beer, is at an average level | 2 (good) |
|                   | corresponds to the type of beer that is the maximum allowable for this type of beer | 1 (satisfactory) |
|                   | does not correspond to the type of beer, lighter or darker than the standard level | 0 (unsatisfactory) |
| **Aroma**         | excellent aroma, corresponding to a particular type of beer, clean, fresh, pronounced | 4 (excellent) |
|                   | good aroma, corresponding to the type of beer, but not sufficiently pronounced | 3 (good) |
|                   | in the aroma noticeable extraneous shades: slightly immature, fruity, very pronounced malty tone | 2 (satisfactory) |
|                   | pronounced extraneous shades in the aroma: fruity, sour, aroma of young beer, etc. | 1 (unsatisfactory) |
| **Taste**         | excellent, full, pure, without extraneous flavors, harmonious taste corresponding to this type of beer | 5 (excellent) |
|                   | good pure taste, corresponds to a specific type of beer, but not very harmonious | 4 (good) |
|                   | not very pure taste, unripe, taste of young beer, caramel taste, weakly expressed | 3 (satisfactory) |
|                   | empty taste and extraneous flavors: yeast, fruit, spicy, sour | 2 (unsatisfactory) |
| **Hop bitterness**| pure hop, soft, harmonious, suitable for the type of beer | 5 (excellent) |
|                   | purely hop, not very harmonious, rough, slightly residual | 4 (good) |
|                   | hop, coarse, residual or weak, which does not correspond to the type of beer | 3 (satisfactory) |
|                   | non-hop, rude | 2 (unsatisfactory) |
It is worth noting that a certain increase in the number of yeast generations caused a slight acceleration of fermentation. Specifically, this trend was observed to \( G_2 \) when fermenting 11 °P wort, to \( G_3 \) for the 12 °P wort, and to \( G_4 \) during the fermentation of high gravity wort (15 °P and 18 °P). At higher values of the generation number, the fermentation slowed down.

### 5.2. Yeast physiological state

When using yeast from previous fermentation cycles, it is necessary to take into consideration the physiological condition of the cells. It is believed that to ensure the proper level of the next fermentation cycle, it is necessary that the content of viable cells exceeds 95 \%, and the content of cells with glycogen is at least 75 \%. It is important that the yeast culture before re-use in the next fermentation contains the maximum amount of intracellular glycogen since its insufficient level leads to incomplete fermentation. In addition, glycogen provides energy to support cells during yeast storage, and, along with trehalose, provides higher resistance to stress, which is especially important when using yeast in high gravity brewing.

Changes in the number of cells with glycogen and viable cells depending on the number of yeast generations are shown in Fig. 3.

It was established that under the conditions of high gravity brewing, yeasts of up to six generations, separated after the fermentation of 15 °P wort, meet the criteria for the physiological state.
5.3. Physicochemical parameters of beer

All samples of beer obtained as a result of fermentation with the participation of yeast of various generations were characterized by very close values of the main physicochemical indicators, including ethanol content, pH, coloration (Table 2).

The ethanol content in beer did not depend on the generation of yeast used for fermentation. Specifically, as a result of the fermentation of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P, the samples of beer with the following ethanol content were obtained: 5.2±0.1; 5.25±0.05; 6.0±0.1; 7.0±0.1 % (v/v), respectively. However, there was a tendency to slightly increase the pH and coloration of beer with an increase in the yeast generation number [16]. Specifically, all beer samples obtained from the fermentation with the $G_7$ and $G_8$ yeast generations had slightly higher pH and coloration values compared to other beer samples.

Table 2. The physicochemical parameters of beer obtained from consecutive fermentations [16]

| Parameter            | Yeast generation | $G_0$ | $G_1$ | $G_2$ | $G_3$ | $G_4$ | $G_5$ | $G_6$ | $G_7$ | $G_8$ |
|----------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Original wort gravity 11 °P | Ethanol content, % (v/v) | 5.2  | 5.1  | 5.2  | 5.1  | 5.2  | 5.3  | 5.3  | 5.2  | 5.2  |
| pH                   |                  | 4.2  | 4.1  | 4.2  | 4.1  | 4.2  | 4.1  | 4.2  | 4.2  | 4.3  |
| Coloration (unit)    |                  | 0.8  | 0.8  | 0.9  | 0.7  | 0.8  | 0.7  | 0.8  | 0.9  | 0.9  |

| Original wort gravity 12 °P | Ethanol content, % (v/v) | 5.3  | 5.3  | 5.2  | 5.3  | 5.3  | 5.2  | 5.3  | 5.3  | 5.3  |
| pH                   |                  | 4.2  | 4.2  | 4.2  | 4.2  | 4.2  | 4.2  | 4.3  | 4.3  | 4.3  |
| Coloration (unit)    |                  | 0.7  | 0.7  | 0.8  | 0.7  | 0.8  | 0.7  | 0.8  | 0.9  | 0.9  |

| Original wort gravity 15 °P | Ethanol content, % (v/v) | 6.0  | 6.1  | 6.0  | 6.2  | 5.9  | 5.9  | 6.0  | 6.0  | 6.0  |
| pH                   |                  | 4.3  | 4.3  | 4.3  | 4.3  | 4.3  | 4.3  | 4.4  | 4.4  | 4.4  |
| Coloration (unit)    |                  | 0.8  | 0.9  | 0.9  | 0.8  | 0.9  | 0.9  | 1.0  | 1.0  | 1.0  |

| Original wort gravity 18 °P | Ethanol content, % (v/v) | 7.1  | 7.0  | 6.9  | 7.0  | 7.1  | 7.0  | 7.1  | 7.0  | 7.0  |
| pH                   |                  | 4.4  | 4.3  | 4.3  | 4.3  | 4.3  | 4.3  | 4.5  | 4.5  | 4.5  |
| Coloration (unit)    |                  | 1.0  | 1.0  | 0.9  | 1.0  | 1.0  | 1.2  | 1.2  | 1.2  | 1.2  |

5.4. Sensory characteristics of beer

The results of studying the sensory quality of all beer samples did not differ significantly and ranged mainly within 18–20 points (Fig. 4). The use of yeast of various generations (from $G_0$ to $G_6$) for the fermentation of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P did not have a noticeable impact on the sensory quality of the resulting beer.

All beer samples with an original wort gravity of 11 °P received an excellent sensory rating. The samples obtained from 12 °P, 15 °P, and 18 °P wort with the yeast of all generations, except $G_7$ and $G_8$, also had excellent quality. Only the yeast of generations $G_7$ and $G_8$ produced beer with a good sensory score.
When assessing the sensory characteristics of beer samples obtained under the high gravity brewing conditions involving the $G_7$ and $G_8$ yeast generations, some deterioration in taste and aroma was noticed. Specifically, in the aroma, there were noticeable extraneous tones – yeast tone, tone of young beer [17]. Some samples also saw deterioration in clarity and color. At the same time, the hop bitterness of the beer did not change.

5.5. Determining the recommended number of yeast generations for their effective use in high gravity brewing

As a result of this study, it was found that the effective fermentation of high gravity wort with a gravity of $15\,^\circ P$ is provided by the yeast up to five generations, $18\,^\circ P$ wort – up to four generations. After the completion of wort fermentation with a gravity of $15\,^\circ P$ and $18\,^\circ P$ in six and four cycles, respectively, yeasts with appropriate physiological properties are obtained, suitable for further use. The fermentation of high gravity wort with yeasts up to six generations produces beer with appropriate physicochemical parameters and high sensory evaluation (Table 3).

Based on the values of the number of generations to meet each of the criteria for the fermentation process and the quality of the beer received, the recommended number of generations for the effective fermentation of high gravity wort has been determined. Specifically, for the $15\,^\circ P$ wort, it is up to five generations, for the $18\,^\circ P$ wort – up to four generations. The number of generations for wort fermentation in traditional brewing is also recommended (Table 3).

### Table 3

| Criterion                        | Maximal number of yeast generations for fermenting the wort of gravity ($^\circ P$) |
|----------------------------------|----------------------------------------------------------------------------------|
| Speed and duration of fermentation | $G_6$  $G_6$  $G_5$  $G_4$                                                      |
| Physiological state of yeast     | $G_5$  $G_6$  $G_6$  $G_4$                                                      |
| Physicochemical parameters of beer | $G_6$  $G_6$  $G_6$  $G_6$                                                      |
| Sensory characteristics of beer  | $G_8$  $G_7$  $G_6$  $G_6$                                                      |
| By all criteria                  | $G_6$  $G_6$  $G_5$  $G_4$                                                      |

6. Discussion of results of studying the impact of yeast generations and wort gravity on the progress of fermentation and the quality of beer

The tendency, established as a result of this research, to a slight acceleration in the fermentation of wort with a gravity of $11\,^\circ P$, $12\,^\circ P$, $15\,^\circ P$, and $18\,^\circ P$ with an increase in the number of yeast generations (to $G_2$–$G_4$) (Fig. 2) can be explained by the adaptation of yeast to the fermentation conditions. Earlier studies, conducted under industrial conditions, showed that the biomass of the second generation of yeast during the fermentation of $15.5\,^\circ P$ wort caused an acceleration of the daily consumption of the extract by $0.2\,^\circ P$ per day [7]. In another study [13], results of the laboratory experiments established that the duration of fermentation decreased with an increase in...
the generation number from $G_1$ to $G_3$ while subsequent generations did not cause further acceleration of the process.

Further increase in the number of yeast generation (more than four generations) leads to a decrease in the fermentation rate (Fig. 2). The reason for this is the changes in the physiological state of yeast, namely a gradual decrease in the number of viable cells and cells with glycogen with an increase in the number of cycles of their serial repitching (Fig. 3). Specifically, only yeasts up to the fourth or sixth generation (depending on the wort gravity) are characterized by a proportion of viable cells exceeding 95%.

The physiological state of yeasts after serial repitching was investigated by the authors of [7]. The cited work shows that after the completion of wort fermentation (the sixth-seventh day) with a gravity of 15.5 °P involving the $G_1$–$G_4$ yeasts at a temperature of 14 °C, the number of viable cells was in the range from 96 to 98%. In another paper [8], it was established that the viability of Pilsner yeast cells decreased from 98.9% at the end of the first fermentation to 95.8% after six fermentation cycles of 12 °P wort by the $G_0$–$G_5$ yeasts at a temperature of 11 °C. However, study [9] has shown no significant changes in the viability of yeast cells even up to ten generations. The number of viable cells has always been more than 98%. However, in this study, the wort was fermented with a gravity of 10 °P and 15 °P at a temperature of 9 °C. Regarding changes in the physiological properties of yeast that occur during serial repitching, it was noticed that mature cells ferment more efficiently and at a higher rate than mixed or young cell cultures [18]. In addition, work [19] found that there is a correlation between the state of the cell surface and the performance of fermentation.

Despite the changes in the physiological state, the various yeast generations used in the fermentation process did not significantly affect the quality of beer (Table 2, Fig. 4). Only beer samples resulting from the yeast fermentation involving $G_7$ and $G_8$ generations had slightly higher pH and color values compared to other beer samples. In addition, when using the yeasts of these generations, some deterioration in the taste and aroma of high gravity beer was noticed. Specifically, extraneous shades were noticeable in the aroma – yeast tone, tone of young beer.

Paper [7] showed that the use of different yeast generations (from $G_1$ to $G_4$) during fermentation did not have a noticeable effect on the sensory quality of the resulting beer. The authors of another work [9] demonstrated that serial repitching of yeast did not affect the total number of higher alcohols in beer and the ratio of the number of higher alcohols to the number of esters. The ability of yeast to produce the correct ratio of flavoring and aromatic compounds remained stable.

The decrease in the sensory quality of beer obtained as a result of the fermentation of wort by yeasts of the seventh and eighth generations (Fig. 4) can be explained by changes in the physiological state of yeast. As a result of a decrease in the viability of yeast (Fig. 3), their autolysis may occur. At the same time, several compounds are released from yeast cells into beer [8]. Among them, ethyl decanoate leads to the appearance of yeast aftertaste in beer, and diacetyl and α-acetolactic acid cause the formation of diacetyl aftertaste or a tone of young beer.

It was found that with an increase in wort gravity, the number of cycles of serial repitching of yeast decreases (Table 3). This is due to the fact that during the production of beer and serial repitching, yeast is affected by various stress factors, which, if they prevail over the protective mechanisms of cells, can impair yeast growth and fermentation performance [20]. Specifically, under the conditions of high gravity brewing, such factors are high osmotic pressure and ethanol concentration formed during fermentation. As a result, during the fermentation of higher gravity wort, the physiological state of yeast deteriorates faster.

Thus, the physiological state of yeast determines the number of cycles of their serial repitching and the quality of the beer received. Since the state of yeast largely depends on the gravity of wort and fermentation parameters, the number of yeast generations that are advisable to use in each individual case will vary depending on the fermentation conditions.

This study identified the recommended number of yeast generations of the Saflaquer W-34/70 race for the fermentation of wort with a gravity of 11–18 °P at a temperature of 12–15 °C.

Further research is required into the influence of yeast generation of other industrially applied races on the productivity of fermentation and the quality of the resulting beer. After all, these process criteria depend not only on environmental factors (wort gravity, fermentation parameters) but are also largely determined by the genetic characteristics of yeast cells.

## 7. Conclusions

1. The stable rate and duration of consecutive fermentations of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P at a temperature of 12–15 °C are provided by yeast up to six, six, five, and four generations, respectively.

2. Yeasts up to five, six, six, and four generations, separated after the fermentation of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P, respectively, in terms of the physiological state, meet the criteria for their further use in the next serial repitching.

3. Production of beer with stable appropriate physicochemical parameters as a result of consecutive fermentations of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P is provided by yeast up to six generations.

4. Beer with stable corresponding sensory characteristics is obtained as a result of consecutive fermentations of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P with yeasts up to eight, seven, six, and six generations, respectively.

5. For the effective use of yeast in consecutive fermentations of wort with a gravity of 11 °P, 12 °P, 15 °P, and 18 °P, it is recommended to apply up to six, six, five, and four of their generations, respectively.

## Acknowledgments

I would like to express my sincere gratitude to the team of managers and employees, especially the microbiologist, at the LLC «Mykulynetskyy Brovar» for the possibility of carrying out the research reported in this paper.

## References

1. Kosiv, R., Kharandiuk, T., Polyuzhyn, L., Palianytsia, L., Berezovska, N. (2017). Effect of high gravity wort fermentation parameters on beer flavor profile. Chemistry & Chemical Technology, 11 (3), 308–313. doi:https://doi.org/10.23039/cciht11.03.308
2. Puligundla, P., Smogrovicova, D., Mok, C., Obulam, V. S. R. (2020). Recent developments in high gravity beer-brewing. Innovative Food Science & Emerging Technologies, 64, 102399. doi: https://doi.org/10.1016/j.ifset.2020.102399

3. Kosiv, R., Kharandniuk, T., Polyuzhyn, L., Palianytsia, L., Berezhovska, N. (2016). Optimization of Main Fermentation of High-Gravity Wort. Chemistry & Chemical Technology, 10 (3), 349–353. doi: https://doi.org/10.23939/chcht.10.03.349

4. Kiněl, T., Dostálek, P., Bráník, T., Olšovská, J. (2021). High-gravity brewing without adjuncts – The effect on beer parameters. LWT, 148, 111755. doi: https://doi.org/10.1016/j.lwt.2021.111755

5. Wauters, R., Britton, S. J., Verstrepen, K. J. (2021). Old yeasts, young beer – The industrial relevance of yeast chronological life span. Yeast, 38 (6), 339–351. doi: https://doi.org/10.1002/yea.3650

6. Kucharczyk, K., Tuszyński, T., Żyła, K. (2018). Effect of yeast harvest moment on a brewing process in beer produced on an industrial scale. Czech Journal of Food Sciences, 36 (5), 365–371. doi: https://doi.org/10.17221/157/2017-cjfs

7. Kucharczyk, K., Tuszyński, T., Żyła, K., Puchalski, C. (2020). The effect of yeast generations on fermentation, maturation and volatile compounds of beer. Czech Journal of Food Sciences, 38 (3), 144–150. doi: https://doi.org/10.17221/193/2018-cjfs

8. Wang, J., Ding, H., Zheng, F., Li, Y., Liu, C., Niu, C., Li, Q. (2019). Physiological Changes of Beer Brewer’s Yeast During Serial Beer Fermentation. Journal of the American Society of Brewing Chemists, 77 (1), 10–20. doi: https://doi.org/10.1080/03610470.2018.1546030

9. Kordilak-Bogacka, E., Diowksz, A. (2013). Physiological state of reused brewing yeast. Czech Journal of Food Sciences, 31 (3), 264–269. doi: https://doi.org/10.17221/84/2012-cjfs

10. Speers, R. A., Stokes, S. (2009). Effects of Vessel Geometry, Fermenting Volume and Yeast Repitching on Fermenting Beer. Journal of the Institute of Brewing, 115 (2), 148–150. doi: https://doi.org/10.1002/j.2050-0416.2009.tb00360.x

11. Piddocke, M. P., Kreisz, S., Heldt-Hansen, H. P., Nielsen, K. F., Olsson, L. (2009). Physiological characterization of brewer’s yeast in high-gravity beer fermentations with glucose or maltose syrups as adjuncts. Applied Microbiology and Biotechnology, 84 (3), 453–464. doi: https://doi.org/10.1007/s00253-009-1930-y

12. Sigler, K., Matouliková, 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 (6), 1027–1035. doi: https://doi.org/10.1007/s00253-008-1830-6

13. Verbelen, P. J., Dekoninck, T. M. L., Van Mulders, S. E., Saerens, S. M. G., Delvaux, E., Delvaux, F. R. (2009). Stability of high cell density brewery fermentations during serial repitching. Biotechnology Letters, 31 (11), 1729–1737. doi: https://doi.org/10.1007/s10529-009-0067-5

14. Slyviak, U., Kosiv, R. (2018). Influence of yeasts generation on the high gravity wort fermentation. 84 International scientific conference of young scientists and students: Youth Scientific Achievements to the 21st Century Nutrition Problem Solution. Kyiv, 28.

15. Slyvyak, U. O., Kosiv, R. B. (2017). Povtorne vykorystannia drizhdzhiv u pyvovarinni. Materialy Mizhnarodnoi konferentsiyi “Dni studentskoi nauky u Lvivskomu natcionalnomu universiteti veternarinoi medytsyny ta biotekhnolohiy im. S. Z. Hzhytskoho”. Ch. 3. Lviv, 166.

16. Slyvyak, U. O., Kosiv, R. B. (2018). Uplyv heneratsiyi drizhdzhiv na orhanoleptychni pokaznyky pyva. Materialy Mizhnarodnoi naukovoi konferentsiyi: Dni studentskoi nauky u Lvivskomu universiteti veternarinoi medytsyny ta biotekhnolohiy imeni S. Z. Hzhytskoho. Ch. 2. Lviv, 193–194.

17. Powell, C., Quain, D., Smart, K. (2003). The impact of brewing yeast cell age on fermentation performance, attenuation and flocculation. FEBS Yeast Research, 3 (2), 149–157. doi: https://doi.org/10.1016/s1567-1356(03)00002-3

18. Smart, K. A., Whisker, S. (1996). Effect of Serial Repitching on the Fermentation Properties and Condition of Brewing Yeast. Journal of the American Society of Brewing Chemists, 54 (1), 41–44. doi: https://doi.org/10.1080/03610470.54.1-44

19. Lin, N.-X., Xu, Y., Yu, X.-W. (2021). Overview of yeast environmental stress response pathways and the development of tolerant yeasts. Syst Microbiol and Biomanuf. doi: https://doi.org/10.1007/s43393-021-00038-4